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THE VALUE AND MECHANISM OF THE COLLOIDAL GOLD TEST.

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THE colloidal gold test of the spinal fluid was introduced by Lange in 1912, and has since been quite extensively employed by German and American workers in the diagnosis of mental and nervous diseases. In this country the test has not come into general use, although it is of considerable value in the routine examination of spinal fluids.

The application of a solution of colloidal gold to the examination of spinal fluid was suggested to Lange (1) by the work of Zsigmondy. The latter had shown that colloidal gold was sensitive to the presence of electrolytes, which caused coagulation and precipitation of the particles of gold, and that this reaction was prevented by the addition to the electrolyte of suitable amounts of various protein substances. These substances, which Zsigmondy called "protective colloids," showed varying degrees of protective action, and under certain standard conditions were specific for each substance. The amount of any protein which was necessary to protect 10 c.c. of gold colloid from the

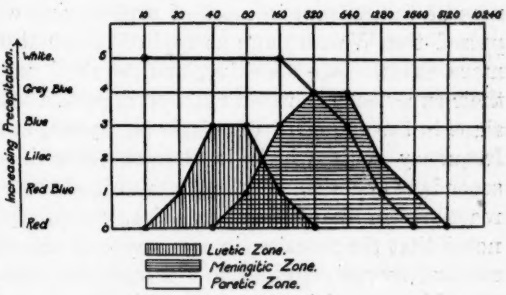
coagulating effect of 1 c.c. of 10 per cent. NaCl was the "gold number" of the protein. It occurred to Lange to examine syphilitic and normal sera by this method, and later to apply the reaction to spinal fluids, as the amount of globulin and albumen was known to vary in different pathological conditions of the central nervous system. Lange found, however, that certain spinal fluids, in addition to exhibiting protective effect on gold colloid, had also unexpected precipitating properties. The spinal fluids of cases of dementia paralytica in particular showed this precipitating property, and as a result of the examination of a series of cases Lange recommended the test for the diagnosis of this disease.

The conditions under which the Lange test is used are different from those which Zsigmondy used in obtaining his gold numbers. In the test of spinal fluid 0·4 per cent. NaCl solution is used, while in Zsigmondy's experiments 10 per cent. NaCl was employed. It will be shown in the course of this paper that the results obtained with strong and weak electrolytes are not connected, the mechanism being quite different in the two cases. This point is mentioned as it is evident from a review of the literature of the Lange test that the reactions have been confused.

The difficulty in applying the gold solution test to routine diagnostic work which all workers have encountered lies in the preparation of suitable samples of colloidal gold. Failures to prepare suitable colloidal solutions have occurred even in the hands of experienced workers, but with the knowledge which has now accumulated the difficulties are easily overcome. Absolutely standard colloidal solutions cannot be made, but practically all specimens can be made of service by simple adjustment of the reaction. It is essential to use pure distilled water, perfectly clean utensils, and pure reagents. The glass-ware should be made of hard glass, and must be thoroughly cleansed with nitro-hydrochloric acid, and repeated washing in distilled and in doubly distilled water. The water must be distilled at least twice over a glass condenser fitted with ordinary corks (not rubber), and should be used soon after distillation. Unsuccessful results are usually attributable to impure water or to imperfectly cleansed flasks. The principle of all the methods is that a weak solution of gold chloride in the presence of weak alkali is readily reduced to metallic gold by the action of certain chemical reducing agents. Any of the reducers in common use for photography are effective, but the best results for the purposes of the test are obtained with formaldehyde. The gold is thrown out in the form of extremely minute particles which, by their dispersion in the fluid, impart to it a deep red colour.

The two methods in common use are: (1) Lange's method, and (2) that of Miller, Brush, Hammers and Felton (2). Lange's method, orginally devised by Zsigmondy, is the simplest and probably the best. The reagents necessary are: (1) 1 per cent. gold chloride solution; (2) 2 per cent. potassium carbonate solution, and (3) 1 per cent. solution of pure formalin. 500 c.c. of doubly distilled water in a clean litre flask are heated to 60 c.c. over a large Bunsen burner, and 5 c.c. of the gold chloride and 5 c.c. of the carbonate solutions added. The temperature is raised rapidly to 90° C., and the flask removed from the flame. 5 c.c. of the formaldehyde solution are added quickly, drop by drop at first, in larger amounts as the reaction proceeds. During the addition of the formaldehyde the flask is very vigorously shaken. A faint pink colour appears which is quickly succeeded by a deep red. The colour is completely developed in less than two minutes. Occasionally the colour is purple or purplish red. These solutions should be discarded. A successful preparation should be a beautiful red fluid, clear by reflected and transmitted light. Fluids with a faint sheen may also be used, but distinctly opaque solutions are unsuitable. Gold solutions produced in this

way show no tendency to settle out even after long periods of time, resist boiling and should be stored in the dark until used. The method of Miller and his co-workers is the same as the above except that 3·5 c.c. of 2 per cent. potassium carbonate are used, and that five drops of 1 per cent. oxalic acid solution are added when the temperature reaches 80° C. At 90° C. 2½ c.c. of 1 per cent. formaldehyde are added drop by drop with vigorous shaking. The colour slowly changes from pink through violet to a deep dark red. This method has been much used in America. According to Black, Rosenberg, and McBride (3), the oxalic acid is not only unnecessary but even inconvenient, in that it neutralises some of the potassium carbonate, which is necessary for the reducing action of the formaldehyde, and results in the production of acid solutions of colloidal gold. These authors have gone minutely into the chemistry of the reaction, and have determined the minimum and maximum amounts of alkali and formaldehyde which may be used. They recommend that the reaction of all samples should be tested, using 1 per cent. solution of alizarin red in 50 per cent. alcohol as indicator. This is done as follows: A double row of tubes is placed in a rack, and 1 c.c. of distilled water added to each tube. 1 c.c. of N/50 HCl is added to the first tube of one series, and 1 c.c. N/50 NaOH added to the first tube of the second series. With a pipette 1 c.c. of the contents of the first tube is transferred to the second tube, and this process of dilution from tube to tube is carried out down each series, the excess of fluid from the last tube being discarded. Two drops of 1 per cent. alizarin red in 50 per cent. alcohol are added to each tube, and 5 c.c. of colloidal gold. The indicator gives a yellow colour in presence of acid and purple with alkali. A red colour with a distinct tinge of brown is regarded as neutral. The amount of acid or alkali necessary to render neutral the whole sample of gold is readily calculated. By adjustment in this way many samples of colloidal gold can be made of service which otherwise would be of little use. It is probable that, if this procedure were adopted generally, results of more uniform character would be obtained. The final test of suitability of any sample of gold is that it should give a paretic reaction with the spinal fluid of a case of general paralysis and show no change with a normal fluid, and that 5 c.c. should be completely precipitated in one hour by 1·7 c.c. of 1 per cent. NaCl solution. In my experience the solutions produced by the original Lange method rarely require adjustment. Solutions of gold which are frankly acid will give reactions with normal fluids, while alkaline ones are not sensitive to paretic fluids.



In performing the test eleven small, dry, clean tubes are placed in a rack. To the first tube 0·9 c.c. of 0·4 per cent. salt solution is added; to the rest 0·5 c.c. A 1 in 10 dilution of spinal fluid is made in the first tube by adding 0·1 c.c. of spinal fluid. This is well mixed and 0·5 c.c. removed and added to the salt in the second tube. The fluid in the second tube is mixed, and 0·5 c.c. transferred to the third tube. This procedure is repeated down the series. In this way dilutions of 1 in 10, 1 in 20, 1 in 40, 1 in 80, etc., are made, the amount in each tube being 0·5 c.c. To each tube 2·5 c.c. of colloidal gold are added. A control tube containing 0·5 c.c. of a 0·4 per cent. salt may be added to the series to serve as a standard of colour. Various types of reaction may occur. These fall into three groups which have been designated the "paretic," "luetic" and "meningitic," or, as Felton and Maxy (4) recommend, zones I, II and III. These terms, though not rigidly descriptive, are so convenient that it is desirable to continue their use. It is customary to describe the changes of colour which occur in the presence of pathological spinal fluids by the numbers 5,

4, 3, 2, 1 and 0. The figure 5 represents a tube in which complete coagulation and precipitation of gold has occurred, leaving the supernatant fluid quite colourless; 4 indicates a pale grey-blue fluid, 3 a deep-blue colour, 2 a reddish violet, 1 a bluish red colour, and 0 indicates no change in colour. In the paretic type of reaction (zone I) complete precipitation of gold occurs in the tubes containing low dilutions of spinal fluid with a gradation of colour changes in the other tubes, *e.g.* 5 5 5 5 4 3 3 1 1 0 0. In the luetic reaction there is little or no change of colour in the first two tubes; the third, fourth and fifth are altered, the maximum change usually occurring in the fourth and fifth, but not exceeding the colour change represented by the figure 4, *e.g.* 0 0 2 4 3 1 1 0 0 0 0. In the meningitic type the colour changes appear in the tubes to the right of the series, the maximum change being in the seventh and eighth tubes and seldom exceeding 3. Complete precipitation of gold in the tubes in this zone has been observed very rarely in cases of meningitis. The paretic reactions are the most striking, owing to the flocculation and subsequent sedimentation of the particles of gold in a number of tubes and alterations in colour in the tubes to the right. In spite of the variation in the methods of preparing colloidal gold which different workers have used, the different zones have been described with considerable uniformity. The text-figure shows the three zones when the results are expressed in the form of a series of curves.

Since 1912 many papers have been published on the clinical value of the gold sol test, particularly in America. The experiences of various observers and the opinions formed regarding the practical value of the reaction may be summarised as follows:

Lange regarded the test as of great value in the detection of early cases of neurosyphilis, and more delicate than the Wassermann. Jäger and Goldstein (5) corroborated Lange's findings in a series of cases of dementia paralytica and cerebro-spinal syphilis. Kafka (6) considered that the gold test was more valuable than any other test of the spinal fluid. Sippy and Moody (7), from the examination of a large series of cases, were impressed with the sensitiveness of the reagent in cases of syphilis and were of opinion that the test surpassed the Wassermann as applied to spinal fluids in that it was qualitatively more exact, more sensitive, and required only a small amount of fluid. In their experience the gold reaction appeared very early and preceded all other signs in the course of dementia paralytica, and its value was enhanced by the frequency with which it showed early involvement of the meninges in secondary syphilis. Eicke (8) examined over 300 cases and obtained typical reactions in dementia paralytica, secondary syphilis and meningitis. He noted that the reaction in tabes was of the luetic type, and recorded a few cases of severe suppurative meningitis in which almost complete precipitation of gold occurred in the meningitic zone. Eskuchen (9) formed the opinion that the test possessed a degree of specificity and sensitiveness not obtained with any of Nonne's four reactions and proposed to call it the "fifth reaction." Solomon and Koefod (10) found that the syphilitic reactions ran parallel with the Wassermann tests of the spinal fluid. Solomon and Welles (11) found that the reaction in tabes was different from that of general paresis and characteristic of syphilis, but were unable to offer any conclusions regarding the value of the test in the differential diagnosis of general paresis and cerebro-

spinal syphilis. Lee and Hinton (12) obtained gold reactions in 24 cases of tabes, of which 9 gave negative Wassermann reactions in both blood and spinal fluid. Of these 9 two gave no other spinal test positive. These patients all had a definite syphilitic history. In 8 cases of syphilis, without clinical evidence of involvement of the nervous system and with negative Wassermann reactions in the spinal fluid, 4 positive gold reactions were obtained. The blood Wassermann was also positive in these four cases. From their findings in a variety of cases they concluded that the gold reaction was nearly constant in syphilis of the nervous system and more delicate than other tests, and that the margin of error was small. No parallelism between the gold tests and the results of any other spinal fluid test was found. Miller and Levy (13) examined 210 spinal fluids from 171 cases, and found that all cases of paresis and tabes gave reactions in the paretic and luetic zones respectively. Luetic reactions were obtained in 13 out of 15 cases of latent syphilis in which there were no clinical symptoms indicating damage to the central nervous system, and in 80 per cent. of cases of congenital syphilis. They were convinced that the test was most valuable in differentiating the various syphilitic diseases of the nervous system, but were of opinion that it had no advantages over other laboratory tests in congenital, secondary, or tertiary syphilis. Further, the test did not distinguish between tubercular and suppurative meningitis. Flesch (14), as a result of his examinations, agreed with the findings of Miller and Levy. Grulee and Moody (15), on the other hand, disagreed with the conclusions of Miller and Levy in regard to the value of the gold test in congenital syphilis, and drew attention to the fact that Miller and Levy had obtained 100 per cent. of positive Wassermann reactions in this condition, a result which is not in agreement with the general experience of laboratory workers. Kaplan and McClelland (16) obtained characteristic curves in general paresis and in tabo-paresis, and luetic reactions in most cases of cerebro-spinal syphilis and tabes dorsalis. Weston, Darling and Newcomb (17), in a large series of mental cases, found the test as valuable as the Wassermann, but obtained "syphilitic reactions" in three cases which gave negative Wassermann reactions in the spinal fluid. In a later communication Weston (18) questions the relation of the gold test to syphilis, and gives details of three mental patients who, during a period of two years under repeated examinations, always gave paretic curves with the gold test and negative Wassermann reactions. In a footnote it is mentioned that one patient, tested while the article was in press, had developed a positive Wassermann reaction. Barnes and Ives (19) and also Swalm and Mann (20) applied the test in large numbers of mental diseases and found it of the greatest value, but did not consider syphilitic reactions were diagnostic of syphilis in the absence of other corroborative tests. Miller, Brush, Hammers and Felton, in a valuable paper, which has already been referred to in regard to the preparation of gold colloid, expressed the opinion

that the gold test was more sensitive and specific in certain conditions than any other tests, and that the simplicity of the technique allowed of easy recognition of the sources of error. They described three cases of disseminated sclerosis giving paretic reactions.

Hammes (21), in a considerable number of syphilitic and other conditions, found no reactions with normal fluids and regarded the test as more delicate than any other. Lowrey (22) obtained no positive reactions in a series of non-syphilitic mental cases, but got 10 per cent. of atypical results. In a communication by Harvey (23) certain cases giving negative results with other tests and positive reactions with the gold test are recorded. Acute poliomyelitis has been examined by Felton and Maxy (24). They report the occurrence of luetic curves in the acute stage of the disease. A recent paper on the subject is that of Warwick and Nixon (25), who have examined 800 spinal fluids from 408 patients suffering from many different nervous and other conditions. These authors find that, while the various curves are not specific, they are of great diagnostic value in conjunction with other clinical and laboratory tests, and that they are of especial value in tabes and cerebro-spinal syphilis. Positive gold tests were obtained more frequently in these conditions than were positive Wassermann reactions. Cases of multiple sclerosis are given which yielded either paretic or luetic types of reaction. Other cases of multiple sclerosis have been examined by De Crinis and Frank (26), Hammes, Miller and his associates, Kaplan and McClelland, Vogel (27), Solomon and Koefod, Flesch, Oetiker (28) and recently by Moore (29). Both paretic and luetic types of curves are recorded, and completely negative results in others. Twenty out of Moore's 28 cases gave paretic curves, with pleocytosis and altered globulin content, but all with negative Wassermann tests. Of the remaining 8 cases 3 gave luetic curves. Moore concludes that these 8 cases are probably not cases of multiple sclerosis and draws attention to the great difficulty in the clinical diagnosis of such conditions. In connection with the occurrence of paretic reactions in this disease it is interesting to speculate upon a possible relationship between these findings and the recent work of Bullock (30), who found that certain cases of multiple sclerosis yielded fluids which were toxic to animals, and the work of Kühn and Steiner (31), Simon (32), Siemerling (33) and Marinesco (34), who report the finding of spirochætes in experimental animals and in man. Another recent study of the colloidal gold reaction is that of Kyrle, Brandt and Mras (35), who have examined 720 cases of syphilis in all stages. They conclude that the gold reaction is specific in so far as it is more pronounced in syphilis than in any other conditions (with the exception of disseminated sclerosis), that it is much less likely to fail or yield doubtful results and that a positive reaction of any kind indicates a considerable change in the spinal fluid even if this is not shown by other reactions. A few other papers dealing with experiences of the reaction are given in local American journals and are quoted by Warwick

and Nixon, but the papers which have been mentioned are sufficient to indicate the scope of the gold test in nervous, mental and syphilitic diseases.

As compared with the large number of cases which have been recorded above by certain workers, the series I have tested is small and confined to mental conditions. Certain observations bearing on the mechanism of the reaction are also presented, which are regarded as of interest in connection with a test which is of value in such a variety of conditions, and the nature of which is so little understood. These observations were for the most part made before the war, and the delay in publication is due to the interruption caused by military service. In the interval some other contributions have appeared which throw light upon the nature of the test and will be dealt with later.

The colloidal gold for these examinations was made by Lange's method, and after a little experience had been acquired no difficulty was encountered in making suitable preparations. Occasionally failures resulted, but these were easily overcome either by redistillation of the water or by greater attention to cleanliness of the glass-ware. Flasks of hard glass, preferably new, gave the best results. Samples of colloidal gold which did not react were rarely met with. These were titrated by the method of Miller, Brush, Hammers and Felton and after adjustment of the reaction were found to be quite suitable. The number of cases examined included 27 cases of general paralysis, 1 of juvenile general paralysis, 3 cases of suspect general paralysis, 1 of combined general paralysis and tabes dorsalis (tabo-paresis), and 58 miscellaneous cases comprising different mental conditions, chronic dementia, epilepsy, dementia præcox, delusional and other insanities. The spinal fluids were drawn off into a series of dry test-tubes and only the fractions which were free from blood to the naked eye were used for diagnostic work. In addition to the gold test the Wassermann reaction was carried out by the method of Browning, Cruickshank and Mackenzie (36). The amount of globulin was tested by the Ross-Jones procedure, also by Noguchi's butyric acid reaction, and in a few cases estimations were also made of the proportion of globulin to albumen. Cell counts of the fluids were not made in all cases and the results, therefore, are not embodied in the tables.

Table I gives the results which were obtained in the cases of general paralysis. They agree with those of other observers in that all cases gave paretic reactions. The Wassermann tests were also positive in all cases and various degrees of globulin increase were detected. No correspondence between the amounts of globulin in the fluids and the reactions with colloidal gold was found.

Table II shows a few cases which gave anomalous results with the gold test or in which there was discrepancy between clinical and laboratory diagnosis. Case 28 was a patient who had been diagnosed as a general paralytic on clinical symptoms. On admission the Wassermann reaction of the

blood was positive, but lumbar puncture could not be carried out owing to the patient's excitable condition. Three months later spinal fluid was obtained and gave a somewhat doubtful positive Wassermann, showed slight increase of globulin, and moderate increase in the number of cells. The gold reaction was not tested at this time. The patient was treated with salvarsan intravenously and subsequently showed improvement in his mental condition. Three years later the spinal fluid was again examined. The Wassermann was negative and the anomalous gold reaction shown in the table was obtained, a reaction which approaches the luetic type but is not typical. As this patient at no time had any symptoms suggesting gumma or localised arterial change, and as he had suffered one congestive seizure in the course of his residence in hospital, the diagnosis of general paralysis was adhered to, but the possibility of cerebro-spinal syphilis could not be denied. Case 29 also presented difficulty in regard to diagnosis. The symptoms suggested general paralysis, but ocular signs were absent and no material change in symptoms occurred over a period of two years. The Wassermann and gold tests were performed on several occasions. The results were always of the kind shown in the table. Cases 30 and 31 are placed together as they were admitted about the same time, having very similar symptoms and both showing left-

TABLE I

Case No.	Wassermann Spinal Fluid.	Gold Reaction.	Globulin.
1	+	55543322100	+++
2	+	55543321000	+
3	+	55555543100	+
4	+	55544321000	++
5	+	55543321000	+++
6	+	54332210000	+++
7	?+	54332221000	+++
8	+	54432100000	+++
9	+	54333210000	+++
10	+	55543210000	+
11	+	55554321000	++
12	+	55532210000	+
13	+	55553210000	+++
14	+	55554321000	+++
15	+	55555421000	+++
16	+	55555421000	+++
17	+	44322111000	+++
18	+	55554321000	+++
19	+	55553221000	+++
20	+	55442210000	++
21	+	55554321000	+++
22	+	45454332100	+++
23	+	55555432100	++
24	+	55543321000	+
25	+	55555544210	++
26	+	45443321000	+++
27	+	55332210000	+++

TABLE II

Case No.	Wassermann Spinal Fluid	Colloidal Gold	Globulin
28	−	04332100000	+
29	+	13221000000	++
30	+	23333321000	++
31	−	11123321000	+
32	+	54433210000	+++
33	−	23221000000	++
34	−	10000000000	?+

sided paresis. Case 30 gave a positive Wassermann and a gold test which approached the luetic in type, and Case 31 a negative Wassermann and a meningitic type of reaction. The gold reaction in the latter was in all probability produced by a recent superficial hæmorrhage with meningeal reaction, as this type is mentioned by some observers as occurring in a few cases of cerebral hæmorrhage. The gold test in Case 30 suggested the diagnosis of cerebro-spinal syphilis rather than general paralysis. Case 32 was a juvenile general paralytic, all the reactions being the same as in the adult type of the disease. In Case 33 the diagnosis of tabo-paresis was made on admission on the basis of symptoms and the finding of a weakly positive Wassermann in the spinal fluid. The gold test was not done. Four years later there was little change in the patient's condition, the Wassermann reaction in the fluid was negative on two occasions, and the gold reactions were as shown in the table. Case 34 was a case with mental changes and nervous symptoms resembling those of multiple sclerosis.

The rest of the cases, comprising fifty-seven in all, and representative of various mental conditions, all gave negative Wassermann and gold reactions, and it is unnecessary to give the results in detail. Some of the fluids, particularly from cases of chronic dementia and epilepsy, showed distinct increase in the amount of globulin comparable to that found in some general paralytics, but caused no alteration in the gold colour. In the group of negative cases slight change in the colour of the gold in the first two tubes was commonly found. In three cases of chronic dementia reactions corresponding to mild meningitic types were found.

The spinal fluid from a young girl with acute meningitis was also tested. This fluid showed only slight increase in protein, contained practically no cells, but was turbid from the presence of pneumococci. The reaction with the colloidal gold was 0 0 0 0 1 1 1 0 0 0 0. The exceptional character of this fluid in respect to almost complete absence of any inflammatory exudate possibly explains the negative result with the gold test.

At the time the above tests were made little was known regarding the gold test beyond the various types of reaction in different diseases. Lange had expressed the opinion that the syphilitic and other reactions were caused by the appearance in the spinal fluid of mixtures of different proteins which were specific for these conditions. Zaloziecki (37), on the other hand, thought that the gold test was an immunity phenomenon. Jäger and Goldstein regarded the reactions as dependent on the electrical changes in the fluid and a purely physical change. Numerous other workers thought that specific proteins were responsible for the different zonal reactions and had obviously confused Zsigmondy's results with protective colloids and the precipitating action of spinal fluid. Matzkiewitsch (38) believed that the reacting substance in the fluids of general paretics was peptone, as he had found that the substance was dialysable and that gold colloid was sensitive to the action of small amounts

of silk peptone. With the exception of this last work no attempt to analyse the various phenomena had been made. In 1916, however, Weston (39) published experiments which appeared to show that the Wassermann reacting substance and the gold reacting substance in paretic fluid were different in that the former was not dialysable while the latter dialysed out quite freely. He also showed that the reaction was probably not caused by the salts of the fluid or by the presence of amino acids. Miller and his associates also had found that the reacting substance was dialysable. In 1917, in a paper read before a meeting of the American Association of Physicans, Felton (40) stated that the reactions were dependent on the relative amounts of globulin and albumen, the globulin exercising a precipitating action and the albumen a protective action. Zonal reactions were due to the inter-relationship of the two substances, small amounts of albumen and globulin giving a reaction in the low dilutions and larger amounts a reaction in the high dilutions. Only a summary of the work has unfortunately been published and no details of experiments are given.

Early in the course of my examination of the gold reaction the association of positive Wassermann tests and paretic curves with colloidal gold suggested that a relationship existed between the two phenomena. As the reacting power of a syphilitic serum or spinal fluid has been shown to reside in the globulin fraction of the protein, paretic fluids were subjected to half saturation with ammonium sulphate in order to throw down the globulin. The precipitate was separated by the centrifuge, washed several times with half saturated ammonium sulphate in order to get rid of albumen, dissolved in salt solution and dialysed till free from salt. The precipitate of globulin was then dissolved in an amount of 0·85 per cent. NaCl solution equivalent to the original volume of spinal fluid. This fluid was tested with the gold test and with the Wassermann. It was found that the globulin had the precipitating power of the original spinal fluid and was even more active. For example, a fluid which gave the reaction 5 5 5 5 4 3 2 1 0 0 0 yielded a globulin solution which gave the reading 5 5 5 5 5 5 4 3 2 1 0. This experiment was repeated with many paretic fluids, always with the same result. The globulin also gave positive Wassermann reactions. Only slight diminution of the precipitating power followed prolonged dialysis of the globulin solutions. Dialysis of paretic fluids in thin celloidin sacs, which were extremely permeable to electrolytes and to peptone, did not appreciably diminish the precipitating action on colloidal gold, nor was any precipitating substance found in the dialysates even when large volumes were tested. Table III shows the results obtained in a typical experiment with a paretic fluid dialysed in a celloidin capsule for twenty-four and seventy-two hours. In each case sufficient 10 per cent. NaCl solution was added to restore the salinity of the dialysed fluid to the equivalent of 0·85 per cent. NaCl solution, as the globulin is only active when in solution.

TABLE III.

Paretic Fluid	5	5	5	5	5	4	3	2	1	0 0
„ „ 24 hours dialysis	5	5	5	5	5	4	4	3	2	1 0
„ „ 72 „ „	5	5	4	3	3	2	1	1	0	0 0

In this experiment amounts of dialysate varying from 1·0 c.c. to 0·1 c.c. all gave no change with colloidal gold. These results are not in agreement with those of Matzkiewitsch, Weston and others, but these workers appear to have used for their dialysis the parchment thimbles which were recommended for use in Abderhalden's pregnancy test, many of which were extremely unreliable. Weston, in fact, found that his capsules permitted the passage of globulin into the dialysates, and these precipitated the gold sol.

The experiments were extended to the examination of spinal fluids which had given anomalous gold reactions. Globulin was separated from the spinal fluid of Case 28, dialysed and tested. A paretic reaction was obtained. Samples of spinal fluid from Case 29 were repeatedly examined in this way. The globulin invariably gave a paretic reaction. The experiments suggested that substances were present in the spinal fluids of these cases which interfered with the precipitating action of the globulin and caused anomalous results to appear. These fluids were therefore again precipitated with half saturated ammonium sulphate and both globulin and albumen fractions prepared and thoroughly dialysed. It was found that the addition of appropriate amounts of the albumen fraction to the globulin interfered with the precipitation of gold and caused anomalous results of the luetic type to appear. The albumen fraction in fact acted as a protective colloid. Other albuminous fluids, which by themselves had no power to precipitate gold colloid, also possessed protective powers. Comparatively small amounts of human or animal serum albumen exerted protective action, and it was possible by adjustment of the amount to render completely negative the action of the globulin on colloidal gold. In many of these experiments it was very difficult to give a reading of certain tubes in the usual numbers as there was frequently distinct turbidity only with no change in colour. The protective effect of albumen was also demonstrated by adding albumen to paretic spinal fluids, the albumen being added to the usual amount of spinal fluid in the first tube only and the appropriate dilutions then made with 0·4 per cent. NaCl solution. For example, the addition of 0·1 c.c. of 1 in 5 dilution of human serum albumen converted a marked paretic reaction into a meningitic one with the reading 0 0 0 0 1 1 3 3 2 1 0.

Cerebro-spinal fluids which gave negative results with the gold test were now examined. It was found that the globulin from these fluids did not precipitate colloidal gold. Even when large volumes of negative fluids were precipitated with ammonium sulphate and the globulin taken up in small volume of salt solution so that the concentration of globulin was from 50 to

100 times that of the original fluid there was only slight precipitation of gold colloid when the concentrated globulin was tested in the usual way. These experiments suggested that the reactions of paretic fluids were not due solely to the increased content of globulin, but to an alteration in the physical or chemical character of the globulin, and that the different zone reactions were dependent on the relative amounts of globulin and albumen, as Felton has suggested. As it has been shown by several workers that the relative amounts of albumen and globulin in the spinal fluids from cases of syphilitic and other diseases of the nervous system vary within wide limits, the possibility of different types of reaction with colloidal gold resulting from such variations is considerable. The way in which the protective action of albumen is exerted is difficult to understand. The protective effect is not shown on the tubes with greatest dilution of spinal fluid, but most markedly on the tubes of low dilution—that is to say, the zone reactions move from left to right of the series, not, as might be expected, from right to left. In fact, by gradation of the amount of albumen, paretic reactions may be converted into luetic, meningitic or negative reactions. Zone effects may also be demonstrated if varying amounts of paretic fluids are added directly to colloidal gold without the use of salt as diluting fluid. Table IV shows the results obtained in this way with various spinal fluids, two of which are paretic, one from Case 29 and the other a fluid giving a negative reaction.

TABLE IV.

(In each tube 2·5 c.c. of gold colloid.)

	Amounts of cerebro-spinal fluid in c.c.					
	0·05	0·1	0·25	0·5	1·0	2·0
Case 25	5	5	0	0	1	1
,, 20	2	2	5	2	3	2
,, 29	0	0	0	1	2	4
,, 59	0	0	0	0	1	1

It will be seen that complete precipitation of 2·5 c.c. of gold colloid occurs with 0·05 c.c. of the spinal fluid of Case 25, whereas no change is produced by the addition of 0·5 c.c. of the same fluid to the same amount of gold. If the amount of colloidal gold in the tube containing 0·5 c.c. is later increased to ten times (25 c.c.) complete precipitation of this large volume results. That is to say, there is a definite relationship between the amount of colloidal gold and the amount of cerebro-spinal fluid which will cause complete precipitation.

Spinal fluids which are active in precipitating colloidal gold under the conditions of Lange's test also possess protective power under other conditions, and this applies both to the globulin and to the albumen fractions. To demonstrate this it is necessary to use an amount of strong electrolyte which by itself precipitates colloidal gold. If to a series of tubes, each containing 0·15 c.c. of 10 per cent. NaCl solution, graded amounts of a paretic fluid are

added it will be found on adding 2·5 c.c. of colloidal gold to each tube that the tubes with large amounts of spinal fluid show no precipitation, while the tubes with insufficient fluid show the colour change and precipitation produced by this amount of salt. Differences in the protein content of spinal fluids can be elicited in this way, those with most protein showing the greatest protective effect. Table V shows the results of such a test.

TABLE V.

(In each tube 0·15 c.c. of 10 per cent. NaCl and 2·5 c.c. colloid.)

	Amount of cerebro-spinal fluid in c.c.								
	0·06	0·05	0·045	0·04	0·035	0·03	0·02	0·015	0·01
Case 12	0	0	0	0	1	2	4	5	5
Case 60	1	2	3	3	4	4	5	5	5

By varying the strength of electrolyte it is possible to show in one series both the precipitating action and the protective one. The protective effect of a cerebro-spinal fluid or serum albumen is not destroyed by boiling, but the precipitating power is completely abolished. If the coagulated protein is removed by filtration or by centrifuging, the protective effect of the fluid is not lessened and may even be increased.

As serum albumen acts protectively it would be expected that the presence of blood in spinal fluid would interfere with the gold test. If the fluid is a paretic one the presence of considerable amounts of blood does not mask the reaction. Slight discolouration of the tubes in the luetic and meningitic zones occurs with negative fluids and no reaction of this type is of value in diagnosis if blood is present in the fluid. Fresh human serum precipitates colloidal gold in high dilutions. Half a cubic centimetre of 1 in 300,000 dilution may produce complete coagulation and precipitation of 2·5 c.c. of gold colloid. A dilution of 1 in 100 serum, used in place of spinal fluid in the ordinary test, will produce a typical paretic reaction. Zone phenomena are frequently encountered in the use of serum, but it is common to find complete sedimentation produced by the use of 0·5 c.c. of all dilutions of serum down to 1 in 300,000. A number of human sera have been examined in this way. Slight variation in activity exists in the sera of different persons, but there is no quantitative difference between syphilitic and normal sera in this respect. As in the case of spinal fluid the reacting substance resides in the globulin. With the globulins zone phenomena are seldom encountered. The albumen fraction of serum has strong protective powers, but has no precipitating property. A 1 in 1000 dilution of serum used in the ordinary test causes the production of a typical meningitic reaction, and it seems probable that the reactions of this type in acute and sub-acute meningitis are caused by leakage of serum proteins through the inflamed meninges. It would be interesting to separate the globulins from meningitic spinal fluids and compare the activity with those of normal and paretic fluids and to determine the degree of

protective action exerted by the albumen fractions. Human serum quickly loses its activity when kept at room temperature under sterile conditions and within a week may have no precipitating action. Nevertheless, the globulins prepared from such old sera are as active as the fresh serum or fresh serum globulin. Human serum differs from most animal sera in its effect on colloidal gold. Sera from the rabbit, guinea-pig, sheep, horse, ox, fowl, mouse and monkey, have all been tested and little or no alteration of gold colloid is produced. The serum of the rat, however, precipitates gold, though it is less active than that of the human subject. This reaction of different sera is at present under examination.

The change which results when paretic fluids or human sera are added to colloidal gold is different from that which is produced by electrolytes, peptone, or other chemical reagents. With the latter the change of colour to deep blue is immediate and precipitation of gold is rapid. In the case of paretic fluid and serum there is little change of colour, the fluid becomes turbid, the particles of gold settle out slowly and the deposit is reddish-violet in colour, not blue, as is the case with electrolytes. The particles of gold in a solution of colloidal gold are negatively charged and will be flocculated by positive colloids and by the cations of electrolytes. The coagulating power of an electrolyte for a negative colloid varies with the valency of the cation and the higher the valency of the cation the smaller is the amount of electrolyte required to bring about coagulation and precipitation. In the performance of the gold test the amount of spinal fluid used is small and precipitation occurs in such a high dilution that it is extremely improbable that the precipitating substance is an electrolyte. Only an electrolyte of high valency could produce the effect, and it would require to be attached to the globulin fraction in such a way that it is not freed by dialysis. It has been demonstrated that, when flocculation is caused by electrolytes, the particles of colloid unite with the ion of opposite electric charge and that the sediment is composed of both substances. An analysis of the sediment which results when a paretic fluid is mixed with gold colloid would determine the nature of the substance causing precipitation. In a series of experiments on this point I have found that, by adding an amount of spinal fluid directly to gold colloid so that complete precipitation just results, the clear supernatant fluid shows complete absence of globulin, whereas control experiments, using the same amount of spinal fluid in a volume of salt solution equal to that of the gold colloid, show the presence of globulin. This experiment supports the other findings that the substance concerned in the precipitation of gold is the globulin fraction of the fluid. The reaction of paretic fluids is removed by shaking the spinal fluid with such substances as kaolin and fuller's earth. This is due to the removal of protein by adsorption. The substance which reacts with colloidal gold resists prolonged exposures to temperatures which fall short of causing coagulation of the protein.

Paretic fluids which are kept under sterile conditions do not lose the precipitating property, and in this respect differ from serum. One author found that a spinal fluid was as active after a year as in the beginning. This is of great advantage, as the same fluid may be used repeatedly in order to test different samples of gold colloid. Spinal fluids obtained post-mortem give the same reactions as ante-mortem fluids.

Samples of colloidal gold made by other methods—for example, by effecting reduction with dilute tannic acid or by a solution of phosphorus in ether—have been tested and found to be unsuitable for the test of spinal fluid. Such colloids are precipitated by paretic and by normal fluids. After prolonged dialysis against pure distilled water these colloids give the same reactions as gold colloid prepared by the use of formaldehyde.

The addition of small amounts of acid to paretic fluids increases the zone of precipitation, while the addition of alkali decreases it. Acid solutions of gold tend to show precipitation with negative fluids and distinctly alkaline ones are not sensitive to paretic fluids. The importance of adjusting the reaction of each sample of gold for use in the test is evident.

SUMMARY AND DISCUSSION.

The results of other workers regarding the value of the gold test in the diagnosis of cases of general paralysis have been confirmed. No parallelism between the gold reactions and those of other tests has been found. The substance in the spinal fluid of general paralytics which causes precipitation of colloidal gold is not dialysable, and resides in the globulin fraction of the protein, and in this respect resembles the Wassermann reacting substance. The substance is not altered by prolonged heating at temperatures short of those which cause coagulation of the protein. No evidence has been obtained which supports the view that the reaction depends on the presence of peptone. The various types of reaction can be simulated by mixtures of globulin and albumen, the globulin acting as a precipitating agent and the albumen as an inhibitory or protective one. Luetic reactions are due to an amount of albumen sufficient to obscure partially the precipitating effect of the globulin. As the globulins obtained from negative spinal fluids, even when used in concentrated form, are almost inactive, the precipitating action of paretic fluids cannot be ascribed solely to the increase in globulin, but is probably dependent on a specific alteration of the physical state of the globulin, which is associated with a positive electric charge. This alteration in the globulin cannot be regarded as specific for syphilis, since it occurs, according to the work of others, in multiple sclerosis, but it is interesting that the change is so constant in one spirochætal disease and common in another which is now suspected to be due also to a spirochæte. The test might serve to separate cases of true multiple sclerosis from those diseases which clinically simulate it very closely. Human

serum globulin also possesses the characters of a positive colloid, but the paretic and luetic reactions of spinal fluids cannot be attributed merely to the passage of serum globulin into the spinal fluid. The meningitic reactions, on the other hand, may be due to the leakage of plasma through meninges damaged by inflammatory change, and are probably of quite a different order to those of syphilis and multiple sclerosis. In reviewing the results obtained by other workers it is evident that for the most part difference of opinion exists in regard to the interpretation which should be put upon reactions of the meningitic type. The fact that this type occurs in such a variety of conditions supports the view that it is dependent on meningeal irritation, which may be caused by tumours, hæmorrhage or inflammatory conditions. The impression has been formed that for the present only reactions of the paretic and luetic types should be regarded as of real diagnostic value, and that the meningitic reactions, while being of some value in conjunction with other findings, should for the present be held *sub judice*.

CONCLUSIONS.

1. In general paralysis the results of the colloidal gold test coincide with those given by the Wassermann reaction. There is a considerable degree of probability that in those cases in which the colloidal gold is positive with a negative Wassermann the gold test is the more delicate indication.

2. No parallelism between this test and the other spinal fluid tests has been found.

3. The reacting substance in paretic fluids is not dialysable and resides in the globulin.

4. The reaction in syphilitic diseases of the central nervous system depends on a physical change in the globulin associated with increased positive electric charge.

5. The zone reactions depend on the relative amounts of globulin and albumen, the globulin having precipitating action and the albumen a protective one.

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THE EXPERIMENTAL TRANSMISSION OF *ENCEPHALITIS LETHARGICA* TO A MONKEY.*

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Soon after v. Economo (1) had drawn attention to the prevalence of *encephalitis lethargica* in Vienna in 1917, v. Wiesner (2) with material obtained from v. Economo's cases, produced a hæmorrhagic encephalitis in a monkey.

The monkey was inoculated subdurally with 0·2 c.c. of a brain emulsion from a fatal case of encephalitis. The effects produced were briefly as follows: The animal recovered completely from the operation, but a few hours later it was noticed to have become very quiet and to sit on the floor of the cage. Next morning it remained in a corner of its cage with its eyes usually shut, and it appeared to be "somnolent." It responded, however, to calls and would still eat. But left alone it shut its eyes and slumbered uninterruptedly. The picture was exactly that of a person struggling to prevent himself falling asleep. There was slight paresis of the right hind foot. Later in the day the sleepiness and the feebleness had markedly increased. The walk had now become ataxic. Death occurred next day, forty-six hours after the inoculation. For some hours previously the animal had been completely apathetic, had lain on the floor of the cage with closed eyes, and had no longer responded to stimuli. At the necropsy, sections of the brain showed a picture typical of acute hæmorrhagic encephalitis affecting the whole cortex and medulla. There were also considerable œdema and hyperæmia. A diplococcus was isolated from the brain. No detailed account is given of the microscopical changes produced. The writer considered the macroscopic changes to be sufficiently diagnostic. Filtered emulsions of the above monkey's brain and emulsions of pure cultures of the pleomorphic coccus failed to produce any fatal illness or symptoms of note in other monkeys.

More recently Strauss, Hirshfeld and Loewe (3) claimed to have successfully inoculated monkeys with brain emulsions and nasal washings from cases of encephalitis.

In their first experiment a *Macacus rhesus* monkey was inoculated subdurally with a saline emulsion of an encephalitis brain. Next day the animal was found to be ill and the spinal fluid contained ninety mononuclear cells per c.mm. Two days later a necropsy showed

* A note on this experiment appeared in the Forty-eighth Annual Report of the Local Government Board, 1918–1919. Medical Supplement. London, 1919, p. 76.

multiple hæmorrhages in the brain, a moderate meningitis and a perivascular infiltration with mononuclear leucocytes. Monkey No. 2 was inoculated with a brain emulsion from this monkey No. 1. The animal showed six days later a hemi-paresis on the side opposite the inoculation. From the appearances seen at the necropsy the authors suggest that the lesion was probably traumatic. Monkey No. 3 was also inoculated with material from the first monkey but the result was quite negative. Monkey No. 4 was inoculated with the filtered washings of the naso-pharynx from a case of encephalitis. The inoculation was made subdurally and intra-peritoneally. Eight days later there was paresis of both hind legs, and the animal was apathetic. The animal recovered completely. Monkey No. 5 was inoculated as above with naso-pharyngeal washings. The day after inoculation there were observed a right hemi-paresis and a paresis of the external rectus of the left eye. Three days later the animal developed a right-sided Jacksonian epilepsy which ended in stupor. The condition got worse, and the monkey was killed on the sixth day. The necropsy showed a condition similar to that of monkey No. 1, and a large hæmorrhage in the pons. Monkey No. 6 was used as a control, and monkey No. 7 was inoculated with material from monkey No. 5. In monkey No. 7 there was persistent paresis of the right arm and leg for two to five days after the inoculation. The necropsy showed a condition very similar to that of monkey No. 1, and in addition a large hæmorrhage with necrosis at the site of the peritoneal inoculation.

In a later communication (4) these writers state that they have transferred the disease to rabbits. The short incubation period in their monkeys, and the type of lesion produced, suggest that these authors were dealing with some virus other than that of *encephalitis lethargica*. Thus, in our experience, gross hæmorrhages have not occurred in cases of human *encephalitis lethargica*. Their experimental lesions resemble those obtained by v. Wiesner, and more recently in rabbits by Sherwood and Downs, with a strain of pleomorphic streptococci. The fact that Strauss, Hirshfeld and Loewe failed to obtain any bacterial growths does not entirely exclude infection by pleomorphic streptococci, because v. Wiesner pointed out that it was only by the employment of special methods that he succeeded in growing his cocci from infected material. Until the lesions produced and the clinical histories of the cases furnishing the material are given in greater detail, the claims of the above authors to have produced *encephalitis lethargica* in animals must be accepted with reserve.

In a previous communication (6) dealing with a number of bacteriological and experimental investigations on *encephalitis lethargica* an account was given of attempts to transmit the disease to monkeys. Material from eight fatal cases, which had shown what are generally considered to be characteristic features of the disease, both from the clinical and histological aspect, was inoculated intra-cerebrally and intra-peritoneally into *Macacus rhesus* monkeys and rabbits. With one exception no definite paralysis was observed in the monkeys, even after an interval which might be considered as a very long incubation period. One or two of the monkeys, however, some ten days after the inoculation showed some degree of excitement and trembling such as are frequently observed before the onset of experimental poliomyelitis. These signs passed off in a day or two and the animals regained their usual vigour.

The one exception was a monkey which developed a flaccid paralysis of the hind legs eighty-four days after inoculation. The paralysis gradually became complete and appeared to be similar to that observed in experimental poliomyelitis. The necropsy showed, however, that the paralysis was due to pressure from a tuberculous granuloma of the lumbar vertebræ.

Since the above attempts several more have been made, but without result until the present recorded instance.

The successful inoculation was obtained with material derived from a case which occurred in a small epidemic in a Home for girls at Derby. For the material and the notes on the epidemic we are indebted to Dr. A. S. MacNalty, who furnished us with a full account of his investigations, of which the following is an extract.

HISTORY OF EPIDEMIC AT DERBY.

At the time of the outbreak there were resident in the Home eighteen girls and a staff of three women. All the girls were said to have been in robust health before the outbreak; none had been attacked by influenza during the epidemic of that year. The majority of the girls were engaged in laundry work, the remainder in housework.

The outbreak commenced on August 14th, 1919. When Dr. MacNalty visited the Home on August 20th all three of the female staff and eight of the girls had been affected. The illness had commenced on the following dates: August 14th, two cases; August 15th, two cases; August 16th, four cases; August 17th, two cases; August 19th, one case. A twelfth case was reported after Dr. MacNalty's visit.

In analysing the clinical features of the outbreak Dr. MacNalty divided the cases into two groups.

Group I.—Abortive Cases.

Of these there were three. The patients were the members of the staff. Their ages were: 55, 34 and 30 years. The symptoms were slight, comprising nausea or slight vomiting and diarrhœa, "fainting turns" and stiffness in the muscles of the neck. One patient had slight bilateral ptosis, most marked in the left eyelid. With the exception of the ptosis, which was still present in this case on August 20th, a slight degree of prostration was the only sequel of a single day's illness. One of the patients remained in bed on the day of illness. The other two continued at their work.

Group II.—Manifest Cases.

Of these there were, up to August 20th, eight cases. The patients were all girls domiciled in the Home. Their ages were: 15, 16, 17, 17, 17, 17, 18 and 22 years. The symptoms and signs were as follows:

Temperature.—The temperatures were not recorded accurately or systematically. An initial, slight rise of temperature seems to have been present, ranging from 99° F. to 101° F.

The symptoms at onset were: Soreness or stiffness of the throat, nausea, in two or three cases actual vomiting, inability to see properly, drowsiness and vertigo, and, in two cases, nose-bleeding.

Ptosis and ophthalmoplegia.—All the cases had bilateral ptosis, and widely dilated pupils with sluggish reaction to light and accommodation. The ptosis was typically progressive and unequal; in the earlier cases it was extreme, in the later it was less.

Other paralyses.—In three cases the palatal reflex was lost, and there was a paralysis of the larynx, which caused complete or almost complete aphonia. The sphincters were not involved. There was neither retention nor incontinence of urine.

Peripheral reflexes.—These were exaggerated. Kernig's sign was present in nearly every case.

Rigidity and spasticity of the lower limbs was conspicuous in one case.

Tremors and irregular jerking movements of the limbs were displayed by two cases.

Voice.—The majority of the patients had a monotonous, nasal voice with a tendency to slurring speech.

Lethargy.—Seven out of the eight cases were, or had been, definitely lethargic. On August 20th, in three cases the patients were in a semi-comatose condition and the stupor had greatly intensified; in two cases the patients were still very lethargic; in one case the lethargy was diminishing; in one case lethargy had deepened into coma, and the patient had died.

In the eighth case the patient had been drowsy, but not definitely lethargic. This case was, in general, mild in type. The pupils were only slightly dilated, and loss of vision had ceased to trouble the patient.

In all the cases examined on August 20th, the patient could be roused out of her lethargy and would answer questions; if left alone, she soon relapsed into stupor. Three of the patients displayed emotional symptoms when roused.

Facies.—All the cases exhibited a facies devoid of expression. One patient, who was seriously ill, had a bright pink flush on the malar bones, and showed champing movements of the jaws.

Fatal Cases.

Of these eight cases in which the illness commenced between August 14th and August 19th, four terminated in death. The age, date of onset of illness and date of death were as follows:

S. A—, aged 17 years. Onset, August 14th; death, August 22nd, 1919.
G. P—, aged 18 years. Onset, August 15th; death, August 25th.
V. S—, aged 17 years. Onset, August 16th; death, August 25th.
M. C—, aged 17 years. Onset, August 16th; death, August 19th.

No cases of *encephalitis lethargica* or of infantile paralysis had been recently notified in the district, nor had the Medical Officer of Health heard of any cases of obscure illness.

The above extract from Dr. MacNalty's full investigation of the outbreak should suffice to demonstrate the accuracy of his conclusion that "*from clinical examination the cases appear to be identical with cases of encephalitis lethargica.*"

The brains from the patients M. C— and S. A— were sent whole in glycerine to the London Hospital. Unfortunately both Dr. McIntosh and Dr. Turnbull were absent on holiday at the time. The brain from M. C— was delivered to the Pathological Institute. It had been in glycerine for twenty-four hours. Dr. W. W. Woods, having immediately removed and placed in 4 per cent. formaldehyde portions for microscopic section, sent the brain to the Bacteriological Department, but when subsequently examined it was considered to be too far decomposed for the purpose of inoculation. A complete history was obtained by Dr. MacNalty of the illness of the patient from whom this brain came. This history and the result of the histological examination of the brain will be given in detail in a subsequent paper. The signs of illness were disturbance of vision, a dry feeling in the throat and mouth, inability to swallow, and increasing lethargy, which gave place to restlessness and emotion when the patient was roused. The illness was more rapidly fatal than in any of the other cases, death occurring within a few hours more than three days after the onset. No active inflammatory reaction was present in microscpic sections of the right frontal, parietal and occipital lobes, the anterior and posterior segments of the mid-brain, the upper extremity of the pons and the upper extremity of the cervical cord.

TECHNIQUE OF TRANSFERENCE OF THE VIRUS TO ANIMALS.

The brain (Case S. A—) from which the material used for the inoculations was derived had been placed in 33 per cent. glycerine and despatched to the London Hospital. But, as already stated, owing to its arrival during the holidays the complete brain had been allowed to remain in the original glycerine for about fourteen days. The brain was found to be very soft. Pieces of the cervical cord, pons and basal nuclei were removed and placed in fresh glycerine for inoculation into animals. Portions of the cervical cord and pons were at the same time placed in formaldehyde solution for histological examination, but the mid-brain was considered to be much too soft for this purpose.

In the *microscopic sections* of the pons and upper extremity of the cervical cord there were no hæmorrhages and no trace of active inflammatory reaction.

For the experiments two monkeys were used: a *Macacus rhesus* and a *Cercopithecus patas*. Both monkeys were inoculated intra-cerebrally and intra-peritoneally. Pieces of the cervical cord, pons and basal nuclei, each about the size of a large pea, were placed in a conical glass vessel, and, after the excess of glycerine had been washed off with sterile saline, were pounded up by means of a glass rod. The pounded material was then emulsified in 20 c.c. of sterile saline. About 10 c.c. of this emulsion was diluted with saline until it could be drawn through a Berkefeld filter with the aid of an exhaust pump. The *Macacus rhesus* was injected with the unfiltered emulsion and the *patas* with the filtered.

SYMPTOMS AND LESIONS OF THE EXPERIMENTAL ANIMALS.

Briefly the effects produced were as follows:

(1) MACACUS RHESUS.

19/9/19.—Received $\frac{3}{4}$ c.c. of the unfiltered brain emulsion intra-cerebrally and 5 c.c. intra-peritoneally. This animal had been observed for some time before the experiment and was known to have a cough, but owing to the absence of others it was necessary to use it.

25/9/19.—Nothing abnormal to date.

2/10/19.—Tendency to be drowsy. This drowsiness gradually increased and the animal at times could only be roused by pulling its hair.

9/10/19.—Condition similar, but animal much weaker. No evident paralysis. Killed by chloroform.

Necropsy.—Subcutaneous fat very scanty. Brain pale; slight increase of subdural fluid. Cord pale; increase of cerebrospinal fluid, in which only a few lymphocytes found.

Lungs: Tuberculous broncho-pneumonia of right lung with a tuberculous abscess (tubercle bacilli present). Left lung, apart from slight œdema at the base, seemed normal.

The abdominal organs (liver, spleen and kidneys) appeared quite healthy. The mesenteric glands were slightly enlarged, and one or two showed commencing caseation.

The brain on section showed nothing abnormal, and pieces were removed and placed in 33 per cent. glycerine and in formaldehyde solution.

Histological examination.—Sections of the mid-brain, pons and cervical cord were examined. No inflammatory infiltration was present. In the mid-brain there were one or two small hæmorrhages. The nerve-cells showed some degeneration, but not sufficient to accept as proof of neuronic degeneration before death.

(2) PATAS MONKEY.

19/9/19.—Received 1 c.c. of the filtered emulsion intra-cerebrally and 5 c.c. intra-peritoneally.

25/9/19.—Monkey had been quite well until the forenoon of the 25th; it then had a fit. After a short interval the animal recovered, but was very tremulous and excited. The temperature was 102·2° F.

26/9/19.—Animal quite recovered.

9/10/19.—No further symptoms; animal appeared to be quite well.

6/11/19.—A severe fit in which the animal was almost rigid and lay at the bottom of the cage in a dazed condition.

7/11/19.—Animal much better; eat well, but unsteady on legs.

10/11/19.—Lay down in cage except when roused.

11/11/19.—Condition very similar, still considerable rigidity and trembling. Disinclined for food, and was frequently to be found with head bent, as if dosing, in a corner of the cage.

12/11/19.—Condition similar.

13/11/19.—Condition similar.

14/11/19.—Died in the afternoon. Placed in ice-chest over-night.

15/11/19.—*Necropsy*.—Nothing abnormal was found in any of the internal organs. Sections of the brain showed some congestion of the grey matter, but nothing of any diagnostic importance. Pieces of the cord, pons and basal nuclei were placed in 33 per cent. glycerine for inoculation purposes; the remainder of the central nervous system was placed in formaldehyde solution for histological examination.

28/11/19.—An emulsion of the cord and basal nuclei was inoculated into three monkeys (two *Macacus cynomolgus* and a *rhesus*), but as no effects were produced the animals, together with a *Cercopithecus callitrichus* monkey, were reinoculated fourteen days later. These animals are still under observation, and one of the *cynomolgus* monkeys has developed symptoms not unlike those of the *patas* monkey.

HISTOLOGICAL EXAMINATION OF THE PATAS MONKEY.

In order to obtain material for inoculation and rapid microscopic examination, a sagittal section was made through the brain, 1 cm. to the left of the great longitudinal fissure. From the left segment thus removed the left basal ganglia together with the greater part of the left centrum semiovale was scooped out. The greater part of the pons and a few portions of the spinal cord were also excised.

A horizontal segment of the left basal ganglia, a vertical segment of the upper part of the pons, and a segment of the cervical cord were immediately placed in 10 per cent. formaldehyde solution, and next day were embedded in paraffin. The remainder of the central nervous system was placed over-night

in the ice-chest, was next day placed in 10 per cent. formaldehyde, and was later transferred to 10 per cent. saline formaldehyde.

The detection of specific changes in the horizontal section of the left basal ganglia led to an examination of the remainder of the central nervous system. In the following account of the microscopic findings the two portions removed for provisional examination are, for greater convenience, placed in sequence of situation among the portions examined subsequently.

The tissue was embedded in paraffin. Sections were stained in Ehrlich's hæmatoxylin with eosin, in Weigert's iron-hæmatoxylin with van Gieson's mixture, in Weigert's fuchselin with neutral red, by Gram's method with neutral red, in the Unna-Pappenheim mixture, and in Jenner's stain.

(1) *Right frontal pole.*—The vessels of the meninges are congested; many are partly occupied by hyaline thrombus. Around one or two vessels in the pia-arachnoid there is a very slight infiltration with lymphocytoid cells.

The vessels in the cerebral substance are also congested, and a few contain hyaline thrombus. The great majority of the pyramidal cells are slightly shrunken and deeply stained. The nucleus is stained deeply, and shows no internal structure apart from a nucleolus. It is central, or slightly eccentric. The cytoplasm is conspicuously dark. It is occupied by a deeply stained net, enclosing pale vacuolar spaces. Forming part of the net neuro-chromatic bodies can very occasionally be recognised. The net appears, therefore, to have been formed by the neuro-chromatin.

The remaining pyramidal cells are larger. The nucleus is large; the threads of chromatin are slender, feebly stained and separated so far as to form a widely meshed net; the nuclear membrane is very slender, pale and indistinct. The nucleus is central, or slightly eccentric. In the feebly stained cytoplasm is a widely meshed net of slender, slightly darker neuro-chromatin. In a very few cells neuro-chromatic bodies can be recognised as forming part of this net, where it occupies a dendron. Glial nuclei occasionally project into bays in the surface of both the dark and the pale cells.

(2) *Right, central, parietal cortex*, and (3) *Right occipital cortex.*—The changes are very similar, save that there is no meningeal infiltration. In the parietal cortex the deeply stained pyramidal cells do not preponderate so greatly over the pale; in the occipital cortex the pale cells are in excess of the deeply stained.

(4) *Coronal section through the left hemisphere in the anterior part of the frontal lobe.*—The pyramidal cells are almost all of the deeply stained type.

(5) *Coronal section through the left hemisphere at the level of the central parietal and temporal lobes.*—Hyaline thrombi in meningeal arteries and veins are numerous. There is a trace of infiltration with lymphocytoid cells in a few portions of the pia-arachnoid. Deeply stained pyramidal cells are not so numerous as in the previous section. In the pale cells traces of neuro-chromatic bodies are more frequent.

(6) *Coronal section through the left hemisphere in the region of the occipital lobe.*—Pale pyramidal cells are much more numerous than dark. In these pale cells evidence of degeneration is less, traces of neuro-chromatic bodies being more frequently present.

(7) *Horizontal section through the left basal ganglia and insula (segment removed for provisional examination).*—The section is at a low level. It includes the putamen and globi pallidi, part of the insular cortex, the external capsule, a large extent of the internal capsule, within which are small trabecular extensions from the head of the caudate nucleus, and a small portion of the subthalamic region.

In the posterior part of the convex outer border of the putamen there is a slight *infiltration* within, and immediately around, the adventitia of two large veins. A similar infiltration surrounds a capillary in the external capsule close to these veins.

In the subthalamic region several vessels show a similar, slight, intra- and peri-adventitial

infiltration. In this region there is also a conspicuous, small, focal area of infiltration which does not surround a vessel.

The cells of the adventitial infiltrations measure from 5 to 6 micra in diameter. Their cytoplasm is basophil. Their nuclei measure about 4 micra in diameter, and contain numerous large deeply stained nodes. They are similar to the cells which are described in greater detail in Section 9.

The cells in the focal area of extra-adventitial infiltration have oval, and bent oval, nuclei, which in structure resemble those of glial cells.

No granular leucocytes were detected in the adventitial sheaths or in the glia.

Many of the glial cells throughout the section show karyorrhexis.

In the nerve cells of the caudate nucleus the cytoplasm is usually pale; neuro-chromatin is usually absent, but is sometimes present in the form of pale granules, or a fused ring, in the periphery of the cytoplasm. The nuclei are almost all pale, and show no structure save a nucleolus and a few scattered droplets of chromatin; rarely the nucleus is pyknotic.

The small cells of the putamen are pale. The cytoplasm contains no neuro-chromatin The nuclear membrane is seldom visible, and the nucleus is usually indicated only by a nucleolus and a few beads of chromatin.

In the large cells of the putamen and globi pallidi the nuclei are usually pyknotic. The neuro-chromatin usually forms swollen, often fused, masses, but it is sometimes powdered, sometimes confined to the periphery of the cells, and sometimes absent. A few of these large cells are very pale, vesicular, and without a nucleus.

The great majority of the cells in the subthalamic region have a pale, vesicular cytoplasm in which a few minute pale granules of neuro-chromatin are occasionally visible. A few cells have pyknotic nuclei and darker granules of neuro-chromatin.

(8) *Vertical section through the basal ganglia.*—The section includes the right basal ganglia and insula, the body of the left caudate nucleus, the inner six-eighths of the left optic thalamus, the optic chiasma and the corpus callosum.

The meninges, choroid plexuses and cerebral substance are congested. Hyaline thrombi are again present in many vessels. There are three small *hæmorrhages* into perivascular canals in the right globus pallidus, and two in the left ansa peduncularis.

In the mesial cortex of the right hemisphere the majority of the pyramidal cells are of the deeply stained type described above. This type of cell is less numerous in the insular cortex. The large cells in the right and left caudate nuclei, in the right pulvinar and globi pallidi, and in the nuclei of the optic thalami are less deeply stained. Their nuclei show a distinct structure. Their cytoplasm is usually vacuolated, and seldom shows a trace of neuro-chromatin. The periphery of the cytoplasm is frequently fragmented.

(9) *Section through the anterior extremity of the mid-brain.*—The section includes the pineal and mammary bodies, the cephalic extremity of the right anterior corpus quadrigeminum, the right pulvinar, the right internal and external geniculate bodies, the substantia nigra, and the anterior extremity of the oculomotor nuclei. On the right side the section extends to, and includes, the cerebral cortex. The left side is terminated laterally, at the outer margin of the crus, by the sagittal section which was made in order to obtain material for inoculation and provisional microscopic examination. The structures on the left side lie slightly nearer the cerebrum than on the right.

In the right lateral geniculate body the cytoplasm of the cells is devoid of neuro-chromatin, is vacuolated, and so fragmented in the periphery that the greater part of the nucleus frequently lies in an unoccupied portion of the perineuronal space. The cytoplasm of the cells in the median geniculate body is more deeply stained, less vacuolar and less fragmented. In the oculomotor nuclei a few cells are pyknotic. The majority are pale, their cytoplasm is structureless, their nucleus is frequently swollen and sometimes eccentric, whilst the periphery of the cell body is occasionally fragmented.

In the substantia nigra the neuro-chromatin is collected into large, often confluent, masses in the periphery of the cells. The nuclei are so pale, dropsical and ill-defined that they are difficult to detect. They are frequently eccentric.

In all the above sites glial nuclei frequently project into bays in the periphery of the nerve cells.

On the left side, at the edge of the coronal section, in the ventral portion of the optic thalamus, in a position immediately cephalic to the lateral geniculate body, is *a group of vessels surrounded by cellular infiltration.* Some of the infiltrating cells lie within the adventitial clefts or between the endothelium and adventitia, but the majority lie immediately external to the adventitia. The vessels are thus surrounded by conspicuous rings of infiltration. Relatively few infiltrating cells lie scattered through the glia at a distance from the perivascular rings. In this area degeneration of nerve-cells does not appear to be greater than in other parts of the section.

A capillary, immediately beneath the ventricular surface on the left side of the pineal body, is surrounded by similar infiltration.

The *cells* in the perivascular rings measure from 5 to 6 micra in diameter, the nuclei being about 4 micra in diameter. The cells are rounded, but one axis is usually longer than the other. The cytoplasm is decidedly basophil, but in the great majority of the cells is less basophil than that of a plasma cell. The nucleus is, as a rule, slightly eccentric. It contains conspicuous, large, deeply stained nodes on a widely meshed net of chromatin. Of these nodes one is frequently in the centre, whilst the remainder rest upon the nuclear membrane. Occasionally the nucleus is of the "clock-face" type, the nodes being triangular and resting with their bases upon the nuclear membrane. The latter nuclei resemble, therefore, those of plasma cells, but in the majority of these cells the cytoplasm is not so abundant and not so deeply basophil as in a typical plasma cell, whilst a pale perinuclear demilune is very seldom present.

In the infiltration of the glia, external to the perivascular rings, there are a few cells of the above kind; the majority of the cells differ in having a nucleus with the delicate structure of a glial cell. In a few perineuronic spaces are similar, free glial cells, and pyknotic, often shrivelled, nuclei devoid of cytoplasm (neuronophagia). In the glia are also portions of fragmented glial nuclei. One neutrophil leucocyte was found in the glia; no neutrophil leucocytes were found in the perivascular rings.

Almost the whole of the infiltrated area in this section is included in the field represented in Plate III. (Objective ⅔ inch; ocular Zeiss 4.)

(10) *Section through the anterior corpora quadrigemina and the right internal geniculate body.*—This section includes the nucleus and fibres of the third cranial nerves, the substantia nigra, the red nuclei and the upper motor nucleus of the trigeminus.

The large globular cells of the upper motor nucleus of the trigeminus are deeply stained and contain neuro-chromatic bodies.

In the oculomotor nuclei the cells show relatively little degeneration. They are of good shape and have central nuclei. The majority are pale, and contain a variable number of scattered, neuro-chromatic bodies. These bodies are usually globular. The other cells are smaller and deeply stained. In these the neuro-chromatic bodies are, as a rule, more numerous, are usually smaller, and of more regular triangular or fusiform shape.

In the red nuclei, also, there are both pale and dark cells. The majority are pale. In these pale cells neuro-chromatic bodies are usually scanty, swollen and globular. The dark cells appear to be shrivelled, and neuro-chromatic bodies can seldom be distinguished therein.

In the substantia nigra the neuro-chromatic bodies are confined to the periphery of the cells, and are usually fused. The nuclei are frequently slightly eccentric.

In the internal geniculate body almost all the cells are swollen, pale, vacuolated and devoid of neuro-chromatin.

The tissues are slightly separated by *œdema* in focal areas within the oculomotor nuclei, the red nuclei and the substantia nigra.

(11) *Vertical transverse section through upper part of the pons. (Segment removed for provisional examination).*—The section passes through the aqueduct of Sylvius and the caudal portion of the posterior corpora quadrigemina. In the grey matter close to the mid-line,

between the aqueduct and the dorsal longitudinal bundles, is a paired nucleus of large cells. This nucleus is apparently not the trochlear nucleus, but caudal thereto. The section includes transverse sections of the fibres of the trochlear nerve.

In the nerve-cells of the floor of the aqueduct and of the pons the neuro-chromatin forms ill-defined masses confined to the periphery of the cytoplasm, or impregnates the cytoplasm diffusely. The nuclei are central. In hæmatoxylin and eosin the staining resembles that of imperfectly fixed tissue. Many of the glial nuclei show karyorrhexis.

There is no evidence of inflammatory reaction.

(12) *Section through the anterior extremity of the medulla.*—The section includes the cerebellum. In this there are three nuclei on each side; on analogy with the cat these are the dentate, the combined embolus and nucleus globosus, and the nucleus tecti. Beneath the fourth ventricle is the dorsal vestibular nucleus and a small nucleus mesial thereto. Ventral and slightly lateral to the vestibular nucleus lies a nucleus composed of large cells, apparently Deiter's nucleus. Lateral to this is a second nucleus of large cells, apparently the nucleus of Bechterew. A small portion of the olives is included. Mesial to this, and slightly ventral, above the dorsal border of the trapezoid body, is a small nucleus, probably the accessory nucleus of the sixth cranial nerve.

The majority of the Purkinje cells are large and pale, and contain only a few scattered, usually rounded, neuro-chromatic bodies. The remaining cells are stained deeply; the larger examples have a deeply stained cytoplasm in which neuro-chromatic bodies cannot be recognised; a few smaller examples contain numerous neuro-chromatic bodies of spindle shape.

The cerebellar nuclei also contain both deeply stained and larger, pale cells. In both forms neuro-chromatic granules are almost invariably absent. The pale cells preponderate.

In the dorsal vestibular nucleus, Deiter's nucleus and Bechterew's nucleus and the accessory sixth nucleus, the bodies of the neurons show similar changes, but scattered neuro-chromatic bodies are more frequently seen.

The majority of the cells of the olives contain deeply stained neuro-chromatic bodies; in some cells these bodies are swollen and rounded; in a few they are absent in a perinuclear zone.

In the accessory sixth nuclei, especially in that on the left side, the glia is dissociated by *œdema*.

In the trapezoid body, above the outer extremity of the right peduncle, *a capillary is infiltrated* by cells similar to those seen in sections 7 and 9. The infiltrated cells lie between the endothelium and the adventitia. This vessel is represented in Plate IV. (Objective $\frac{1}{12}$ inch, ocular Zeiss 4.)

(13) *Section through the posterior extremity of the medulla.*—The section includes the following structures: The internal arcuate fibres; three bilateral large dorsal nuclei separated by zones of longitudinal fibres—the mesial of these is obviously the gracile nucleus, the external has very large cells and is presumably the cuneate or accessory cuneate, whilst the third nucleus lies between and slightly ventral to these, and has cells similar to those of the gracile nucleus; the nuclei of the central grey matter; the separated ventral portion of the anterior horn of the cord (nucleus ambiguus); the olives, and mesial olives.

In the gracile nuclei there are a few pyknotic cells on the left side. The remaining cells are pale and swollen; neuro-chromatin is confined to the extreme periphery of the cells in the form of a few small bodies; the cytoplasm is often greatly vacuolated and the nucleus completely lysed. The cells of the intermediate dorsal nucleus show similar changes.

In the lateral dorsal nucleus (cuneate nucleus) neuro-chromatin is in general more abundant, but where most abundant it is scanty and is in the form of rounded bodies of different sizes.

In the central grey matter and in the nucleus ambiguus the cells are pale. Their nuclei are slightly dropsical. Neuro-chromatin is either absent whilst the cytoplasm is vacuolated, or it is confined to the periphery of the cells in small granules or in swollen clumps.

In the olives the neuro-chromatolysis is in general at its least. But it is present in

different degrees, and several cells have lost their nuclei. In the median olives the neuro-chromatolysis is greater.

(14) *Upper cervical cord.*—A few of the cells of the anterior horns contain neuro-chromatic bodies of fairly good shape and distribution. The great majority of the cells are swollen, vacuolated and pale, have a dropsical nucleus, and contain at the most only a few, ill-defined, poorly stained particles of neuro-chromatin. In many cells the peripheral cytoplasm is fragmented by vacuoles; in these cells the nucleus is very difficult to distinguish or cannot be seen. Shrunken pyknotic cells with bays in their periphery are scanty. In the posterior horns pyknotic cells are relatively abundant.

(15) *Cervical enlargement.* (*Segment removed for provisional examination.*)—The sections include a portion of a posterior root ganglion.

The nerve-cells have been fixed imperfectly, but neuro-chromatic bodies can be demonstrated throughout their cytoplasm.

(16) *Cervical enlargement.*—The section includes the nerve roots.

Degenerations similar to those in Section 14 are present, but there are many more cells containing neuro-chromatin. In several cells the neuro-chromatic bodies are deeply stained and evenly distributed.

(17) *Upper thoracic cord.*—Degeneration is greater than in the preceding section. Shrinkage of the neurons, with crenation of their outer borders, is commoner than swelling and vesiculation.

(18) *Mid thoracic cord.*—In the anterior and lateral horns the changes are very similar. In Clark's column the cells contain numerous neuro-chromatic bodies.

(19) *Lumbar enlargement and roots.*—There is a great difference in the appearance of different neurons. All the types of degeneration already described are present. Some cells are obviously swollen; their neuro-chromatin is represented by ill-defined bodies in the extreme periphery of the cytoplasm; their nuclei are eccentric, and the nucleolus therein may lie against the nuclear membrane. Such a cell often lies next a slightly shrivelled cell containing swollen, deeply stained neuro-chromatic bodies. Where neuro-chromatin is abundant, it is almost invariably in the form of bodies of different size and shape.

(20) *Sacral cord with cauda equina.*—Less degenerate cells with evenly distributed neuro-chromatic bodies are more frequent in this segment, but many cells show neuro-chromatolysis, vacuolation, disintegration or pyknosis.

(21) *Lung.*—There is slight anthracosis. The bronchi and bronchioles are healthy. There is catarrhal desquamation of epithelial cells in a few alveoli which lie against bronchioles. There are also a few small areas of collapse. The remainder of the vesicular tissue shows emphysematous distension.

No bacteria are present.

(22) *Liver.*—The liver is engorged.

(23) *Kidney.*—The kidney is engorged. The epithelium of the tubules shows slight cloudy swelling. There are no casts. Active inflammatory reaction is absent.

(24) *Spleen.*—There is a remarkable quantity of iron (Prussian blue reaction) in the form of coarse granules, not only in the pulp but in Malpighian bodies. There is no evidence of inflammation.

Examination of central nervous system for bacteria.—No bacteria were detected within the substance of the tissue or within the vessels. Bacteria were, however, found upon the surface of segments taken from the material which had been placed in the ice-chest overnight before fixation. They were present upon the surface of segment 9, including the surface of the coronal section made by the knife in separating the left cerebral hemisphere. They were also present upon the outer surface of the dural theca of the spinal cord, and of nerve roots outside the theca. The majority of the bacteria were Gram-negative bacilli. With these were associated Gram-negative diplococci.

In this monkey, therefore, no pathological changes of significance outside

the central nervous system were found on examination with the naked eye and with the microscope.

Within the central nervous system active inflammation was present in the posterior part of the left basal ganglia and in the junction of the left basal ganglia with the mid-brain; inflammation represented by a very slight perivascular infiltration of the pia was present over areas of the cerebrum; an inflammatory infiltration of the wall of one vessel was also present in the trapezoid body on the right side of the anterior extremity of the medulla.

The changes other than inflammatory infiltration were: engorgement, hyaline thrombosis, hæmorrhages into "perivascular canals" in the right globus pallidus and left ansa peduncularis, abnormalities in the bodies of neurons, and œdema of certain cranial nuclei. The exact significance of the abnormalities in the bodies of the neurons is very difficult to determine. It must be borne in mind that the animal was found dead in its cage, part of the material was hurried through fixation, and the remainder was left in the ice-chest for two nights before fixation. Bacteria were present upon the exposed surfaces of the latter material. Under these circumstances the bodies of the neurons and their neuro-chromatin could not be expected to be in perfect condition. The severer forms of ante-mortem degeneration and necrosis were not detected. With few exceptions the degenerations were similar to those which have been found in material fixed in formaldehyde solution after removal from cases in which death had been due to accident and the cadavera had been preserved over-night in the freezing-chambers. But there were exceptions. For instance, the degeneration of some of the cells of the anterior horns of the lumbar cord cannot be ascribed to changes after death. Further, these cells were in close proximity to others in which little, if any, degeneration could be detected. Such divergence in the condition of cells in particular nuclei was common, and is in favour of degeneration before death. It can only be said that there was evidence of the occurrence of neuronic degeneration before death, but that the degenerations actually observed cannot be accepted in their entirety as having occurred before death. The cells in which the degeneration appeared to be greater than could be attributed to changes after death, were distributed throughout the central nervous system.

The focal œdematous dissociation of the tissues in certain nuclei cannot be attributed to changes after death. Further, such focal œdema is very common in these very nuclei when infiltrated in infantile paralysis and *encephalitis lethargica*.

CONCLUSIONS.

There can be but little doubt that, in this experiment, *encephalitis lethargica* developed in a monkey after the inoculation of the filtered emulsion from the brain of a human case of this disease. The clinical manifestations in the monkey resembled with considerable closeness those of human *encephalitis*

lethargica. The histological changes reproduced in exaggerated form the chief characteristics of human *encephalitis lethargica*: inflammatory infiltration was very strictly localised, and was confined to the areas chiefly affected in the human disease. The finer histological features were also reproduced: the grosser forms of cellular necrosis were not found; neurono-phagia was very slight; a search with special methods for emigrated granular leucocytes revealed only one such cell.

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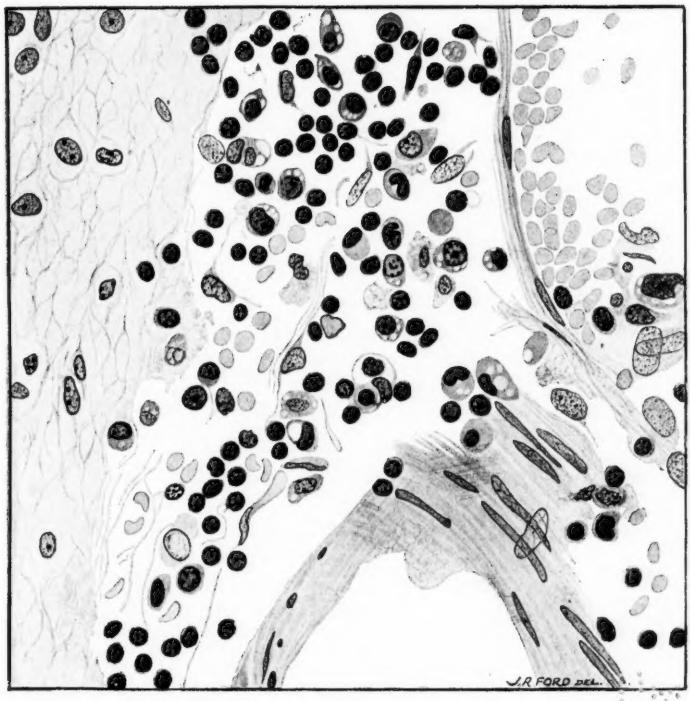
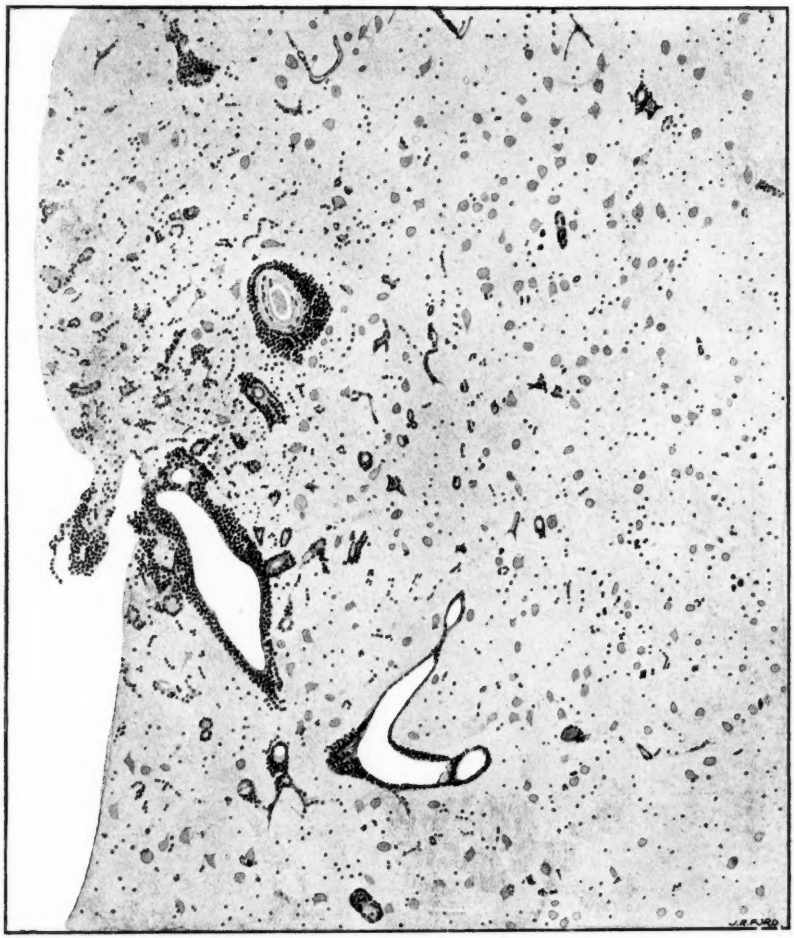
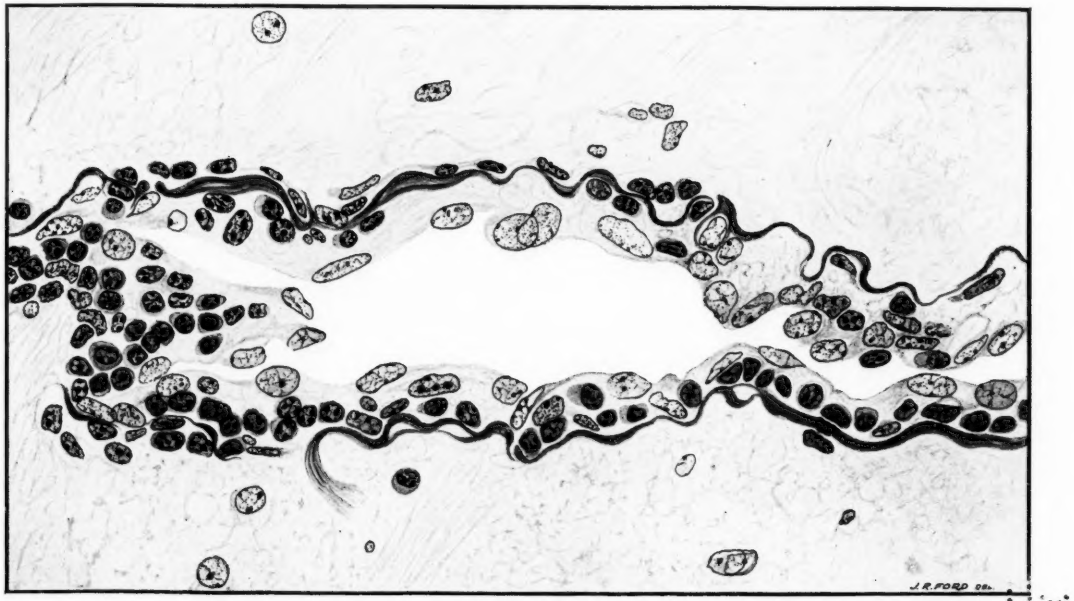
Human case for comparison with Plate III. Man, aged 48 years.
Basal nuclei. Weigert's iron-hæmatoxylin and acid fuchsin.
Obj.: $\frac{2}{3}$". Oc.: Zeiss 4.



Human case for comparison with Plate IV. Man, aged 48 years. Basal nuclei. Weigert's iron-hæmatoxylin and acid fuchsin.
Obj.: 3 mm. Oc.: Zeiss 4.



Patas monkey. Left optic thalamus (Section 9, p. 98). Jenner's stain. Obj.: $\frac{2}{3}$". Oc.: Zeiss 4.



Patas monkey. Right trapezium (Section 12. p. 99). Weigert's iron-hæmatoxylin and van Gieson's mixture. Obj.: $\frac{1}{12}$". Oc.: Zeiss 4.

CONDITIONS WHICH ARE CONDUCIVE TO THE PRODUCTION OF SHOCK BY HISTAMINE.

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DETAILS of the shock-like collapse of the circulation, associated with depression or failure of the respiration, which is produced by histamine in anæsthetised cats and dogs, have been published by P. P. Laidlaw, A. N. Richards and myself (1, 2, 3, 4). The central feature of the condition appeared to be an oligæmia, a diminution of the volume of blood in effective circulation, which we were led to attribute to a loss of the normal tone of the capillary blood-vessels, and a morbid permeability of their endothelial walls. We were interested in this action, produced by an organic base, not merely as a pharmacological curiosity, but because it appeared to represent a type of action common to a large group of poisonous substances of animal or bacterial origin. It had, at the time of our publication, an immediate interest in its resemblance to a shock-like condition prevalent during the war as a sequel to massive injury of the tissues, in which an aseptic toxæmia appeared to be a prominent factor (Cannon and Bayliss, 5), (Bayliss, 6, 7), (Cannon, 8, 9), (Quénu, 10), (Delbet, 11). We believed, moreover, that the type of action would have a more general pathological interest, in that it represented a generalisation over the whole circulatory system of phenomena recognised, when occurring locally, as inflammation, and had therefore an important bearing on the mechanism of the failure of circulation and depression of metabolism associated with many types of rapidly generalised infection or toxæmia.

There has been evident, in certain more recent publications, a tendency to attribute to the resemblance in action a chemical significance, for which we found, and for which I still find, insufficient justification. Histamine, of course, is closely related to and easily derived from the amino-acid histidine; and an attractive simplification could be effected in our conception of many pathological processes if we could assume that, wherever an action of this type is detected, histamine itself has been produced. Abel and Kubota (12), in a highly suggestive paper, have given evidence which leads them to the

conclusion that histamine is produced with great readiness from muscle, liver and other tissues, and is split off at an early stage in the hydrolysis of any ordinary protein. On the chemical side, however, the evidence is still incomplete, and the suggestion that histamine itself, or even a derivative of histamine, is actually formed when a tissue is injured can only be regarded as an open possibility. In dealing with certain influences which have an adjuvant effect on the poisonous effect of histamine, I am, therefore, still regarding this substance simply as a convenient agent for the quantitative production of a certain type of effect. I have already published, in brief summary, a note on some of the factors which contribute to the production of histamine-shock (4). My purpose here is to give a fuller account of the experiments there mentioned and of some others, and to discuss their practical implications.

EFFECT OF ANÆSTHETICS.

Laidlaw and I (2) mentioned the fact that anæsthetisation of the animal (usually a cat) by chloroform, ether or urethane, or by decerebration under a preliminary volatile anæsthetic, was a factor in the production of histamine-shock. We had observed the toleration of relatively very large doses of histamine by unæsthetised cats, and had been led to relate the occasional failure of the shock to appear, in cats under ether, to the rapidity of the preliminary preparation, the animal having been only a short time under the anæsthetic before the dose of histamine was injected. We had not, however, deliberately explored the extent of the tolerance in the normal animal, nor had we eliminated the factor of individual variation of resistance. In the following experiments this has been effected by testing the effect of histamine on the same cat under different conditions, with an interval of some days between the two experiments, to exclude any after-effect of the first:

Cat 1.—February 5th. Experiment without anæsthesia. Weight 2300 grm. Paws unpigmented. The animal was gently secured on a warmed table. A drop of blood from the ear-vein had a hæmoglobin value of 88 per cent.

12.14 p.m.—Begin slow infusion of 0·1 per cent. histamine into the left internal saphena vein.

12.16 p.m.—Cat salivating. Heart-beat slow (vagus inhibition), respiration deep. 1 mgrm. injected.

12.19 p.m.—3 mgrm. injected. Heart still inhibited by vagus, but beats vigorous. Paws and buccal mucosa bright pink.

12.22 p.m.—5 mgrm. injected. Pupils widely dilated.

12.24 p.m.—6 mgrm. injected. Mucous membranes slightly dusky. Heart-beat quicker.

12.28 p.m.—10 mgrm. injected. Condition much the same. Respiration more rapid. Pupils still dilated.

The infusion was continued at about the same rate, with little change in the condition, till 12.43 p.m., by which time 23 mgrm., *i. e.* 10 mgrm. per kilogram, had been injected. The infusion was then stopped and the cat released. It seemed a little stiff in its movements, but stood and walked. Respiration was not perceptibly abnormal and the heart-beat was vigorous. A sample of blood from the ear-vein at 12.50 p.m. showed a hæmoglobin value of

98 per cent., indicating some concentration. The cat soon began to clean its fur, jumped from the table and ran across the room. There was no attempt at vomiting during the infusion; fæces had been passed, but without violent purgation. At 2 p.m. the animal still seemed to be perfectly normal, and a third sample of blood showed return to the initial hæmoglobin value of 88 per cent.

The cat remained in perfect health till February 10th, when the second experiment was performed. The weight was still 2300 grm.

Cat 1.—February 10th. Experiment under ether anæsthesia maintained for two hours before histamine was given.

2.45 p.m.—Cat anæsthetised with chloroform and then kept under light anæsthesia with ether till 4.37 p.m.

4.37 p.m.—Rectal temperature 36·8° C. Ether strengthened till anæsthesia was deep. Tracheal cannula inserted, through which ether was then given. Arrangements for recording arterial pressure and taking samples of arterial blood. Both carotids tied and both vagi cut.

4.54 p.m.—Start record. Blood-pressure 190 mm. Hæmoglobin 75 per cent.

5.2 p.m.—Blood-pressure 160 mm. Hæmoglobin 75 per cent.

5.5–5.6 p.m.—Inject 2·3 mgrm. of histamine (1 mgrm. per kilo.) into femoral vein. Blood-pressure falls rapidly from 160 mm. to less than 40 mm. (see Fig. 1). When the blood-pressure reaches about 45 mm. respiration fails. Artificial respiration applied and continued thereafter.

5.12 p.m.—Inject a further 2·3 mgrm. of histamine, making 2 mgrm. per kilo. in all. The blood-pressure shows a slight temporary rise to 40 mm., and then falls further, the output of the heart becoming so small as scarcely to move the mercury in the manometer.

5.16 p.m.—Blood-pressure 26 mm. Hæmoglobin 118 per cent.

5.24 p.m.—Blood-pressure about 20 mm. Output practically imperceptible.

Thereafter, till the animal was killed at 6.12 p.m., the arterial pressure remained at about 18 mm. The beat of the heart, as felt through the chest-wall, was fairly vigorous, but the output was so small as to cause no visible pulse-waves on the manometer record. By compressing the abdomen, and temporarily driving blood into the heart, the pressure could be raised to about 45–50 mm., and the pulse at once became visible on the record. On relaxing the compression the circulation relapsed to its previous condition.

The post-mortem appearances were typical of histamine-shock as described in our earlier papers. The heart was practically empty.

The contrast between the effects of histamine on the same cat, under the different conditions of the two experiments, was very striking. In the case in which no anæsthetic was administered 10 mgrm. per kilo. were tolerated with but slight and evanescent effects; at the end of two hours under ether, although the animal was apparently in excellent condition, with an arterial pressure of 160 mm. after section of the vagi, 1 mgrm. of histamine per kilo. produced a profound condition of shock, and after 2 mgrm. per kilo. all tendency to recovery vanished. Several other experiments were made in which histamine was similarly infused into a vein without anæsthetic, and the results were, in general, the same. One further such experiment may be summarised.

Cat 2.—Weight 2100 grm. Blood from ear-vein has hæmoglobin value 72 per cent Respiration 56 per minute. 21 mgrm. of histamine (10 mgrm. per kilo.) were injected in 45 minutes. The pupils were dilated in the early stages, and the respiration deep. After 5 mgrm. had been injected the mucous membranes were slightly blue, and the respiration very rapid (140 per minute) and shallow. Thereafter the cat became distinctly narcotised, the

pupils gradually contracted, and the breathing became slow and quiet. If the cat was roused deliberately it panted rapidly with open mouth, but relapsed into stupor with quiet breathing. When the injection was finished the animal lay as if asleep, and could not be roused and made to stand. The hæmoglobin value had risen to 92 per cent. Within an hour the cat had regained its normal activity and the hæmoglobin was 82 per cent. After another hour the animal had perfectly recovered and the hæmoglobin value had returned to the initial 72 per cent.

No experiments were tried with larger doses than 10 mgrm. per kilo., but there seems little doubt that this limit could have been exceeded without fatal or even serious effect.

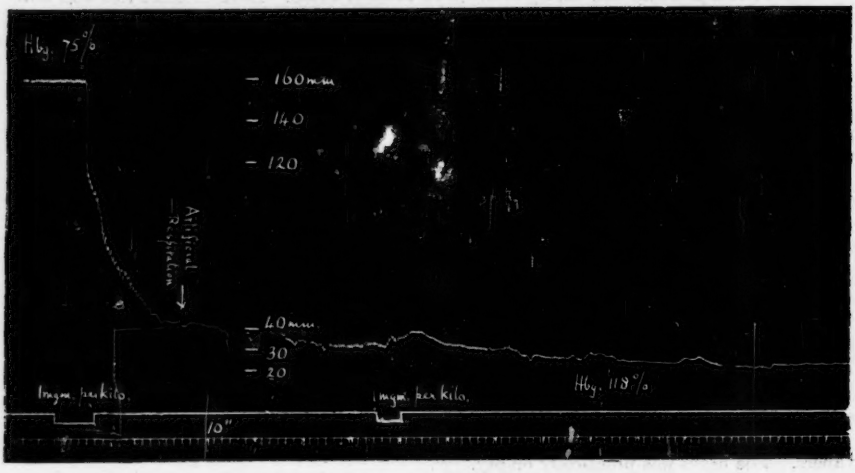

FIG. 1.—*Cat* 1. Effect of 2 mgrm. of histamine per kilo. on arterial pressure, at the end of two hours' anæsthesia with ether.

The experiment on Cat 1 after two hours under ether having been made during continued ether anæsthesia, it seemed desirable to determine whether the effect of the anæsthetic persisted after its removal.

Cat 3, weighing 4400 grm., was therefore kept for 2 hours under light ether and then allowed to recover. Thirty-five minutes after the administration of ether was discontinued the animal was still slightly drowsy, but otherwise normal. 9 mgrm. of histamine (*i. e.* just over 2 mgrm. per kilo.) were then infused into the saphena vein in 15 minutes. Cyanosis of the mucous membranes appeared almost as soon as the infusion was started. When it was finished the animal lay deeply unconscious, with shallow, rather rapid respirations, occasionally interrupted by a deep inspiratory gasp. As there was no sign of recovery a little ether was given —very little sufficing to abolish the corneal reflex completely—tracheotomy was performed, and the arterial blood-pressure recorded. It was about 100 mm., rising to 130 mm. after a deep inspiration, but the pulse was hardly perceptible on the record. The pressure gradually fell away, and spontaneous respiration failed. Artificial respiration was applied, but did not arrest the fall of blood-pressure, which reached 60 mm. after about 20 minutes. On opening

the abdomen the typical appearance in histamine-shock was found—the intestines dusky, with dark venules, the pancreas purple and œdematous, the large veins poorly filled. The heart, though beating strongly, was very empty, and on incision ejected only a few c.c. of blood.

A sample of arterial blood showed a hæmoglobin value of 146 per cent. and a corpuscular content, by hæmatocrit, of 64 per cent. An initial control sample had unfortunately not been taken, but, even if the normal hæmoglobin value were over 90 per cent., which is very unusual in the cat, and the corpuscular content 40 per cent. instead of the average 33 per cent., the concentration caused by histamine would have been of an extreme order.

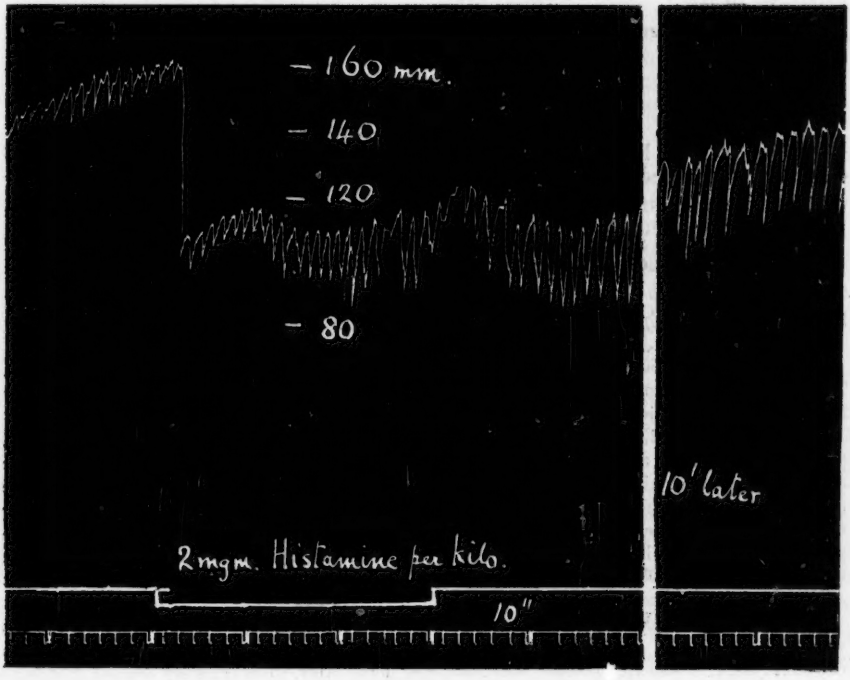

FIG. 2.—*Cat* 7. Effect of 2 mgrm. of histamine per kilo. on arterial pressure, at the end of one hour's anæsthesia with nitrous oxide and oxygen.

It is clear, then, that the adjuvant effect of ether on the shock-producing action of histamine persists after most of the ether has been eliminated from the system.

In another experiment a longer interval of recovery after ether was allowed.

Cat 4, weighing 3200 grm., was kept under light ether for two hours, and then left for two hours, when it had apparently recovered completely. 12·8 mgrm. (*i.e.* 4 mgrm. per kilo.) of histamine were then infused into the saphena vein in eighteen minutes. At the end of the infusion the animal was deeply narcotised, the limbs were rigid, and the respiration took the form of rapid panting, with open mouth and protruded tongue. The hæmoglobin value had risen from the initial 84 per cent. to 96 per cent.

The cat lay unconscious for half an hour, but was sitting up at the end of an hour. The hæmoglobin value was still 96 per cent., and even two hours later the animal still seemed drowsy and weak, and the hæmoglobin value was 98 per cent.

By the next day this cat had completely recovered; but it is clear that the preliminary period under ether, even though its obvious effects had passed off, had so lowered the resistance to histamine that 4 mgrm. per kilo. produced a much profounder and more lasting depression than 10 mgrm. per kilo. usually produces in the perfectly normal cat.

The effect, then, of this group of anæsthetics, under which histamine in small doses produces shock, and of which I have taken ether as the type, appears to be due not directly to the effect of the anæsthetic on the central nervous system, but to a more lasting depression of some mechanism of resistance to the poison. The experience of surgeons at clearing-stations during the war had shown that an onset of fatal "wound-shock" frequently followed the administration of chloroform or ether for some necessary operation, in a patient whose general condition had previously given no cause for anxiety. In such cases anæsthesia with nitrous oxide and oxygen had no such accentuating effect on a tendency to shock, and it was of special interest, therefore, to examine the effect of histamine under this anæsthetic.

Cat 5, weighing 3500 grm., was anæsthetised for one hour with nitrous oxide and oxygen. The proportion was roughly 4 to 1, but to maintain complete anæsthesia it was necessary from time to time to increase the proportion of N_2O, producing a slight cyanosis.

A sample of blood, showing hæmoglobin 78 per cent., was taken at the end of the hour, and under continued anæsthesia with the same mixture histamine was infused at an average rate of 1 mgrm. per kilo. in four minutes. The effects were very similar to those seen in the animal without general anæsthetic. When 2 mgrm. per kilo. had been injected the breathing tended to become shallow, and the proportion of N_2O was progressively weakened as the narcotic effect of the histamine itself became pronounced. By the end of the infusion, when the cat had received 35 mgrm. (= 10 mgrm. per kilo.) in forty minutes, equal parts of nitrous oxide and oxygen were being given.

Reflexes returned almost immediately on removing the administration mask, but the cat was unable to stand. The hæmoglobin value was 100 per cent. An hour later, when the cat could walk, it was still 100 per cent., and two hours later, when recovery seemed otherwise complete, 96 per cent.

Next morning the cat was perfectly well. It had eaten heartily during the night and the hæmoglobin value was 74 per cent. It was again anæsthetised, this time with ether, for a preliminary period of one hour. Then, still under ether, arrangements were made to record the arterial pressure and infusion of histamine begun. By the time 5 mgrm. (= about 1·4 mgrm. per kilo.) had been injected in seven minutes the blood-pressure had fallen from the initial 186 mm. to 44 mm., and the respiration had failed altogether. Under artificial respiration a further 2 mgrm. (making a total of 7 mgrm., or 2 mgrm. per kilo.) reduced the blood-pressure to 41 mm. Hæmoglobin value 106 per cent. After about fifteen minutes a slow recovery of blood-pressure, under continued artificial respiration, became perceptible, and it stood at 60 mm. half an hour after the injection was stopped. It was again reduced to 35 mm. by injection of 1 mgrm. of histamine. On opening the chest the characteristically empty but strongly beating heart of histamine shock was seen. The intestines were duskily congested, the pancreas œdematous. The heart was cut open and the experiment finished.

It will be seen that in this cat 10 mgrm. of histamine per kilo., administered during prolonged anæsthesia with nitrous oxide, produced but little more effect than that produced by the same dosage without anæsthesia; under ether, 2 mgrm. per kilo. produced an intense shock, which would have been immediately fatal but for the application of artificial respiration.

Further experiments were made under nitrous oxide and oxygen, in which the arterial pressure was recorded from the carotid artery.

Cat 6.—Weight 1600 grm. Anæsthesia with N_2O and O_2 for thirty-eight minutes before record started. Blood-pressure 165 mm. Hæmoglobin 74 per cent. 16 mgrm. of histamine injected intravenously in less than twelve minutes. Blood-pressure falls slowly to 100 mm.; then, after 4 mgrm. have been injected, slowly recovers. At the end of the infusion it is 150 mm. Hæmoglobin 86 per cent. Fifteen minutes later blood-pressure 140 mm. Cat killed under anæsthesia.

Cat 7.—Weight 2900 grm. Kept under N_2O and O_2 for an hour before beginning the record of arterial pressure (reproduced in Fig. 2). Vagi cut. Blood-pressure, fluctuating rather widely with respiration, about 160 mm. Hæmoglobin 84 per cent.

Inject 6 mgrm. of histamine (= about 2 mgrm. per kilo.) in three minutes. Blood-pressure falls immediately to about 110 mm.; three minutes later still 110 mm.; hæmoglobin 82 per cent.; ten minutes later blood-pressure 130 mm. Cat killed under anæsthetic.

The contrast between the effects of histamine, under ether and under nitrous oxide respectively, will be sufficiently obvious from a comparison of Figs. 1 and 2. Note that in Cat 7, 2 mgrm. of histamine per kilo. produced no increase in the concentration of the blood.

EFFECT OF PRELIMINARY HÆMORRHAGE.

A large proportion of the sufferers from wound-shock had probably had more or less serious hæmorrhage at the time of the infliction of the wound. The amount of blood lost would seldom be on record, and a calculation based on the corpuscular content of the remaining blood would be vitiated by the tendency of the shock-factor to produce concentration, in place of the normal dilution following simple hæmorrhage. The corpuscular content might seem surprisingly normal, and was often reported as doing so; but the initial hæmorrhage, though its effect on the blood was thus masked, would obviously intensify the severity of an oligæmia subsequently produced by loss of plasma into the tissues and stagnation of blood in the capillaries. Experiment showed that a preliminary hæmorrhage greatly weakened the resistance of the unanæsthetised animal to histamine.

Cat 8.—Weight 2100 grm. The left jugular vein was punctured and about 50 c.c. of blood withdrawn. The hæmoglobin value fell from 86 per cent. to 60 per cent. The cat seemed drowsy and lethargic, but otherwise normal. Two and a half hours later the hæmoglobin was still 60 per cent. Infusion of histamine was then begun into the saphena vein at the rate of 1 mgrm. in about one and a half minutes. The respiration was almost immediately affected and became progressively shallower. When 6 mgrm. had been injected in ten minutes the infusion was stopped. The hæmoglobin had risen to 74 per cent. The respiration continued

to get weaker and failed altogether seven minutes after the infusion was stopped. The heart could still be felt beating vigorously. *Post-mortem* the typical picture of fatal histamine shock was presented.

The preliminary hæmorrhage had so weakened resistance that rather less than 3 mgrm. of histamine per kilo., injected without general anæsthesia, sufficed to produce fatal shock.

EFFECT OF REMOVING THE SUPRARENAL GLANDS.

Richards and I (1) drew attention to the possibility of an antagonism between adrenaline, on the one hand, and histamine and the group of capillary poisons with similar action, on the other. We found that in the artificially perfused organ the capillary tone which histamine relaxes could only be maintained if the perfusing blood contained a trace of adrenaline; so that the presence of the latter was necessary for demonstration of the vasodilator effect of histamine in an artificial perfusion. We suggested that a normal function of adrenaline might be the maintenance of capillary tone against the depressant action of traces of the products of cellular injury or metabolism. It is consistent with that suggestion, though not decisive evidence in its favour, that the cat deprived of its suprarenal glands shows a remarkable lack of resistance to histamine. In these experiments the suprarenal glands were removed with aseptic precautions under ether. Particular care was taken to avoid hæmorrhage and to secure absolute hæmostasis before the wound was closed. On the following day, when the animal had completely recovered from the immediate effects of the anæsthetic, and before serious symptoms due to suprarenal defect had made their appearance, histamine was infused intravenously without anæsthesia. In the earlier experiments, when the extreme sensitiveness to the drug was not expected, a dose probably in excess of that needed for a fatal effect had been injected before the result was realised. The following table shows the doses after which death occurred.

Weight of cat.	Time of decapsulation.	Time of injection of histamine on following day.	Dose after which death occurred.
Cat 9, 2000 grm.	4 p.m.	2.33 p.m.	1·5 mgrm. (= 0·75 mgrm. per kilo).
Cat 10, 2100 „	6 p.m.	11.21 a.m.	1·5 „ (= 0·71 „ „).
Cat 11, 3000 „	11.45 a.m.	9.50 a.m.	0·5 „ (= 0·16 „ „).
Cat 12, 2500 „	12 noon.	12 noon.	1·3 „ (= 0·5 „ „).

Cats 11 and 12 had each, some days before operation, received an injection of 10 mgrm. histamine per kilo. with the usual evanescent symptoms and complete recovery.

Another cat (13) was decapsulated under ether, kept till the following day, and then anæsthetised with ether again and the blood-pressure recorded. The blood-pressure was about 80 mm., the pulse slow, and the beat of the heart, as felt through the chest-wall, vigorous. On section of the vagi the beat became rapid and the pulse waves recorded by the mercury manometer so

small as to be almost invisible. On tying the other carotid artery the pressure rose to 90 mm. and then gradually sank to 58 mm. The picture suggested the presence already of an incomplete "shock" of the histamine type. Injection of 0·01 mgrm. of histamine caused a fall of pressure to 35 mm. There was but slight tendency to recovery from this minute dose, the effect of which in the normal animal is very evanescent. The arterial pressure returned only to 42 mm., respiration failed, and in spite of artificial respiration the pressure gradually fell to 18 mm. Though the manometer recorded practically an unbroken straight line, the heart could be felt beating strongly through the chest-wall, but gradually became weaker and failed altogether 8 minutes later. It was found to be practically empty.

There seems to be a resemblance between the condition of the circulation, which slowly develops after the suprarenal glands have been extirpated, and that which histamine rapidly produces in the animal under ether. The resemblance is emphasised by the appearance of a condition which I have not elsewhere found recorded, but which I have observed in all cases in which I looked for it, namely, the pronounced concentration of the blood which occurs during the period of survival after extirpation of the suprarenal glands. Thus in Cat 11 the hæmoglobin value of blood from the ear-vein rose from 72 to 96 per cent., in Cat 12 from 76 to 104 per cent., in the period of about 24 hours between the extirpation and the fatal injection of histamine. In Cat 13 it was initially 82 per cent., 98 per cent. 24 hours later, and 109 per cent. after administration of ether for the record of blood-pressure, but before giving histamine. All the operations were performed extra-peritoneally, through lumbar incisions, and the appearance of extensive œdema in the retro-peritoneal tissues, discovered *post-mortem*, to some extent accounts for the loss of blood-plasma. The concentration was not proportionate to the extent of this œdema, and cannot, I think, be regarded as wholly attributable to it. On the other hand, there is a suggestive connection between the occurrence of this œdema in the neighbourhood of the operative tissue-injury, the increased permeability of the capillaries in general, and the extreme sensitiveness to histamine which follows the operation. It would seem that the animal, through loss of its suprarenal glands, has already been brought almost to the limit of its resistance to the toxæmia following the inevitable trauma, so that a normally trivial dose of histamine suffices to turn the scale in the direction of fatal collapse.

DISCUSSION.

The practical bearing of these observations is evident. Evidence obtained during the war had pointed to an aseptic traumatic toxæmia, due to absorbtion of substances produced by injury and autolysis of tissues, as a central factor in the condition termed "secondary wound-shock." There were, however,

various accessory and complicating factors—exhaustion, pain, cold, thirst, hæmorrhage, anæsthetics—the precise *rôle* of which was always difficult to estimate. I have examined the effect of certain of these factors, which were suitable for laboratory experiment, and found that they lower in a very striking degree the normal resistance to histamine. It seems probable that the resistance of the otherwise perfectly healthy subject to products of tissue injury would similarly be high, and it is reasonable to attribute the demonstrated danger of chloroform or ether to the severely wounded man, and the relative safety of nitrous oxide, to the much greater effect of the former anæsthetics, as shown in these experiments, in depressing the normal resistance to this type of toxæmia. It seems possible, further, that the greater safety in civilian operations, as shown by Crile and others, of the combination of local anæsthesia with nitrous oxide and oxygen, as compared with prolonged anæsthesia with ether or chloroform, may find its true explanation along the same lines. Certainly, I think, the conclusion is warranted, that in cases in which bacterial or other toxæmia is present chloroform and ether should be avoided whenever possible.

The weakening of resistance by hæmorrhage needs little comment. The necessity of restricting the loss of blood in operation, if shock is to be avoided, has long been recognised. I think it is justifiable to suppose that the significance of the hæmorrhage, like that of the anæsthetics, lies not so much in the harm which it would do by itself, as in its effect of lowering the threshold of tolerance to poisonous products from injured tissues.

The effects of removing the suprarenal glands are at once the most striking, the most suggestive, and the most difficult to interpret. We are dealing with a complex of effects produced by removal of both the cortex and the medulla of the glands. The present trend of opinion seems to be in favour of regarding the fatal effect of the removal as due to lack of the cortex. If we are to regard the extreme sensitiveness to histamine as due to the absence of this part of the gland, the observation can simply be taken at its own value. We can only note the probability that poisonous substances of the histamine type are probably at all times being formed in the body in small quantities; the resistance of the normal animal to their action may, therefore, give a hint of an unknown function of the suprarenal cortex, and of the cause of the death which rapidly follows its ablation. On the other hand, there are points of support for the view that the secretion of adrenaline by the medulla of the gland is, at least, an important factor in the normal resistance to histamine. It is easy to demonstrate that a small dose of histamine accelerates the secretion of adrenaline in the normal animal. In a cat from which one superior cervical ganglion has been removed some days previously, the pupil on the side of the operation has a greatly accentuated sensitiveness to adrenaline. If 0·01 to 0·1 mgrm. of histamine is injected into the ear-vein of such a cat a pronounced dilatation of the sensitised pupil results. The effect is exactly that of injecting

a small dose of adrenaline, or of any influence causing a small acceleration of its secretion from the suprarenal glands.

If the records given above, of the results of infusing large doses of histamine into normal cats, be consulted, it will be seen that wide dilatation of the pupils accompanies the infusion. My experimental records seem to show that when, as in Cat 1, this dilatation is pronounced and persistent, the symptoms produced by the infusion are relatively slight and evanescent, but that when the dilatation is not well marked, and passes off in the later stages of the infusion, the symptoms are more severe and recovery is delayed. Cat 11, for example, a week before the suprarenal glands were removed, received the usual test-infusion of 10 mgrm. of histamine per kilo. My note records that the pupils throughout the infusion showed little dilatation, and that when half the dose had been administered they were not dilated at all. At the end of the infusion the cat was deeply narcotised, and needed some hours for complete recovery. It must be admitted that such observations are suggestive rather than convincing. If we had clear evidence that the extreme sensitiveness to histamine, caused by extirpation of the suprarenal glands, was due to lack of adrenaline, it would be justifiable to consider whether this lack were not the common factor in all the conditions by which such sensitiveness has been found experimentally to be enhanced—anæsthesia with ether, hæmorrhage, adrenalectomy—or which clinical observation has recognised as contributory to the production of shock—exhaustion, excitement, pain. I do not think, however, that the evidence yet available is sufficient to bear this weight of interpretation.

SUMMARY.

Histamine can be given by intravenous infusion to a normal cat, not under an anæsthetic, in doses up to at least 10 mgrm. per kilo., without severe or lasting effect.

In the same animal, at the end of anæsthesia for an hour or more with ether, 1 to 2 mgrm. of histamine per kilo. will produce the characteristic fatal "shock." Anæsthesia with nitrous oxide and oxygen has not this effect of lowering the resistance to histamine.

A large but not in itself serious hæmorrhage greatly reduces the resistance to histamine.

On the day following operative removal of the suprarenal glands the resistance of cats to histamine was found to be reduced to a very low level, as little as 0·16 mgrm. per kilo., given without anæsthetic, having proved fatal in one instance.

The circulatory condition developing in the twenty-four hours following removal of the suprarenal glands has features in common with that produced by histamine, including a pronounced increase in the proportion of corpuscles in the blood.

The bearing of these observations on the nature of the conditions conducive to the production of traumatic shock is discussed.

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THE COMPARATIVE OXYGEN AVIDITY OF NORMAL AND MALIGNANT CELLS MEASURED BY THEIR REDUCING POWERS ON METHYLENE BLUE.

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It has been known since the work of Ehrlich (1) that fresh tissues and, in some circumstances, extracts of tissues possess the power of decolourising methylene blue in the presence of water. Schardinger (2) found that fresh milk would rapidly bleach solutions of methylene blue, provided that an aldehyde such as acetaldehyde or formaldehyde was present, and that this property was destroyed by boiling. Bach (3, 4) showed that an extract could be prepared from such tissues as liver and kidney which would reduce methylene blue in the presence of acetaldehyde, and this enzyme apparently reduced nitrates to nitrites also.

Some time ago it occurred to me that a measure of the relative oxygen avidities of normal and malignant cells might be obtained by observing the amount of methylene blue which such cells would reduce. It has been found that both normal and tumour cells will live for some time in Locke's solution, and this fluid, omitting the glucose, was therefore used as the diluent throughout these experiments. At first varying concentrations of methylene blue were tried, varying from 0·2 per cent. to 0·001 per cent. Of these the most suitable strengths were found to be either a ·001 per cent. or a ·002 per cent. solution of the dye in Locke's fluid. I have found it convenient to use special test-tubes for these experiments, each tube being 20 cm. long by 0·5 cm., the capacity being 10 c.c. The tissues are removed with aseptic precautions, and are minced either with scissors or with the mincer designed by Haaland. Weighed or measured quantities (0·3–0·5 c.c.) of the tissues to be studied are placed in these tubes by means of a glass syringe and long hollow needle, and the tubes filled up with a measured amount of the methylene blue solution. A layer of paraffin is poured on to the surface to prevent re-oxidation of the methylene blue, and the tubes are then incubated at 37° C. The effect noted is a progressive decolourisation of the blue fluid, beginning at the bottom next the tissue, and gradually extending upwards. Readings are

made either every half-hour, or hour, up to five or six hours. A reading consists of measuring with a millimetre rule the height of the column of methylene blue decolourised by the tissues. This may be quickly and accurately done by means of a special rack designed for these tubes.

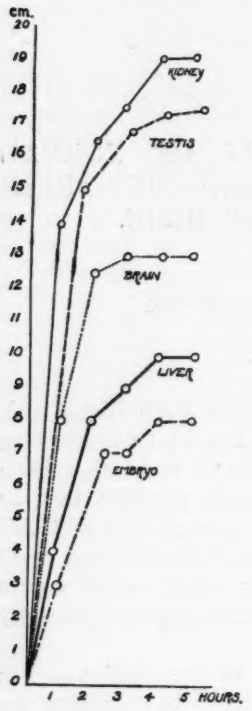

FIG. 1.—Curves of decolourisation of methylene blue by normal tissues.

A great variety of normal mouse tissues was first examined in the manner described, including kidney, liver, spleen, testis, heart, brain, mammary gland, connective tissue and embryo. The height of the column of methylene blue reduced, though varying, is very similar for the normal tissues, brain and spleen giving the lowest readings, and kidney and testis the highest. In order to compare the results from different experiments the heights of the columns of decolourisation were plotted as ordinates against the time as abscissæ. When this is done all the normal tissues give curves of the same general character (Fig. 1). The curve rises steeply from the start, and gradually slows down in the second, third and fourth hours, after which only minimal quantities of dye are bleached.

Having examined many normal tissues in the manner described, a great number of times, I next examined a number of transplantable mouse carcinomata (Jensen, Twort, 63, 113, 206), a mouse sarcoma (37 S.), a rat sarcoma (Jensen), and a scirrhus mammary carcinoma from the human subject. A very striking difference became apparent with all these tumours, when compared with normal tissues. Thus, taking kidney as an example of a normal tissue, we find, on examining the curve produced, a great rise in the first hour, followed by smaller but continuous rises up to the fourth or fifth hours, when reduction usually ceases. In a tumour, however, such as Jensen's adeno-carcinoma, the curve is quite different. No apparent reduction takes place for the first hour or two. A small rise then takes place, ceasing, usually, about the fourth or fifth hour, when transplantation experiments show

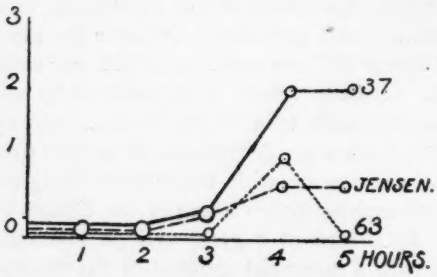

Fig. 2.—Curves of decolourisation of methylene blue by mouse tumours.

the cells to be dead, and microscopical examination shows that the mitochondria have become either very degenerate or have disappeared altogether. Fig. 2 shows curves obtained from Jensen's adenocarcinoma, 37 S. and 63, whilst Fig. 3 shows a curve from 113. All these tumour curves appear to be very similar, the only one showing a striking difference being 113. This tumour is particularly interesting as it contains considerable amounts of glycogen. Amongst all these tumours, leaving aside the exceptional case of 113, those showing the greatest reducing powers are 37 S. and 63, and this is interesting as both are of rapid growth.

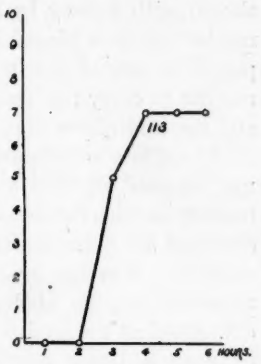

FIG. 3.—Curve of decolourisation of methylene blue by a glycogen-containing mouse tumour (strain 113). Apart from the initial lag, this tumour approaches closely to the behaviour of normal tissues.

Cramer (5) showed some little time ago that if tumour cells were shaken with a soluble ionisable calcium salt a profound depression was produced, and the percentage of "takes" on transplantation fell considerably. Tumours exhibiting this artificial depression could, however, be restored by shaking with sodium salts. This depressant action of calcium salts is very well shown by testing tissues, such as kidney, with methylene blue, after shaking with a soluble calcium salt such as the nitrate. Fig. 4 illustrates this action well. The curve is taken from kidney, which had been shaken for fifteen minutes with a M/7 solution of calcium nitrate. It will be noticed that the resulting curve is very similar to those obtained with tumours, there being the same initial delay. Fig. 4 also illustrates the restorative action of sodium salts, the kidney being shaken with M/7 sodium chloride, after the treatment with calcium. The curve obtained is a normal one.

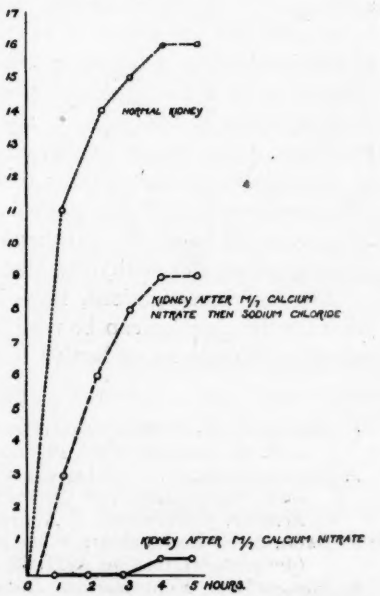

FIG. 4.—Depressant action of calcium salts on the reducing power of kidney, and restoration of this power by washing out the calcium with sodium chloride solution.

That the living cell is necessary for this reaction is shown not only by the effect of calcium salts, but also by the fact that if the tissues be frozen and ground up till no intact cells can be demonstrated by the microscope, very little reduction takes place. This may be conveniently

shown with kidney by finely mincing the organ and then grinding up in a mortar which is placed in a freezing-mixture. The necessary amount is then placed in one of the tubes, filled with Locke's fluid, and centrifuged for a minute to carry the fine particles down, the supernatant fluid is pipetted off and the methylene blue solution added.

If a dilute solution of hæmoglobin be made in Locke's fluid the reduction may be read off with a direct vision spectroscope and recorded in the same manner as with the methylene blue, and if this be done it is seen that the curves obtained are quite similar to those obtained by means of the methylene blue reaction. Further investigation showed that the apparent lag exhibited by all tumours can be abolished if the methylene blue solution be thoroughly exhausted at the pump and washed with pure hydrogen three or four times, exhaustion being carried out till the solution boils between each hydrogen wash. Normal tissues placed in such hydrogen-washed fluids reduce the blue at a very rapid rate and to a greatly increased amount, whilst tumours, although reducing much less than the normal tissues and not nearly so rapidly, show no lag as measured by the hourly readings.

It would appear to be reasonable to conclude that the reduction of the methylene blue is a measure of the oxygen taken up by the cell as the process appears to follow the hæmoglobin curve. Methylene blue, however, contains no oxygen, and the reduction to the leuco-base is entirely due to the addition of nascent hydrogen atoms to the molecule, the hydrogen apparently being obtained from the intracellular water whilst the oxygen is utilised by the cells. The lag which is noticeable in the tumours is probably due to the fact that they have a low oxygen avidity and that the slight amount of oxygen dissolved in the solution is enough for their needs for some time. If, however, the solution be exhausted and washed with an indifferent gas, then, there being no free oxygen to hand, the cells have to obtain it from the solution and consequently reduce the methylene blue.

Further investigations, now in progress, will be required to determine whether the reaction can be used to distinguish neoplastic from other pathological proliferations, or benign from malignant tumours.

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CRITICAL REVIEW.

THE ÆTIOLOGY OF INFLUENZA.

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THE recent epidemic of influenza witnessed a great revolt against the view, up to then generally accepted, that the organism, *B. influenzæ*, discovered by Pfeiffer in 1892, was the cause of the disease.

An opposition to the Pfeiffer discovery had, indeed, developed very soon after its publication and had been maintained intermittently to the time of the recent epidemic. This opposition was based upon the fact that *B. influenzæ* was so often absent from small outbreaks of influenza which occurred after the discovery of the bacillus, while, on the other hand, it was found in large numbers in other outbreaks. Furthermore, the bacillus began to be described in other diseases which had no connection with influenza and even in normal persons. Thus it was not clear upon what grounds it should be considered the cause of influenza any more than the streptococci or pneumococci which were frequently found in this disease and were equally wide-spread.

In spite of these objections, the majority of bacteriologists supported the view that *B. influenzæ* is the cause of influenza, although it may be admitted that a considerable proportion had little or no experience of this organism in its relation to the epidemic disease.

When the recent pandemic began to assume serious proportions bacteriologists set themselves to find *B. influenzæ* with the methods described by Pfeiffer and it is now common knowledge that they largely failed. It is thus not surprising that the anti-Pfeiffer school received many adherents.

Very soon the cause of influenza began to be ascribed to certain invisible or almost invisible bodies, and at one time these "filter-passers" were widely regarded with favour.

Meanwhile the arguments of the more cautious bacteriologists that *B. influenzæ* was after all nearly always demonstrable in influenza if proper methods were used, received scant attention. Since Pfeiffer, in 1892, had laid such stress upon the enormous numbers of bacilli present and the purity and copiousness of his growths, it was thought idle to plead that success depended upon the use of some newly-discovered culture medium.

Following the epidemic a large amount of work was carried out upon the ætiology of influenza, without, however, reaching a conclusion. This work showed that the Pfeiffer school was far from being disposed of; in fact an increasing mass of material was collected which favoured *B. influenzæ*, while, on the other hand, little advance was made in the filter-passing concept of the disease.

It may be remarked that much bacteriological work upon the ætiology of disease suffers from the drawing of speculative conclusions and a disregard of logic in deducing the value of experimental results. Thus in making an attempt to review the claims of *B. influenzæ* on the one hand and filterable viruses on the other as the immediate cause of influenza, it will be necessary to notice shortly the fundamental grounds upon which the causation of a disease may be ascribed to a presumed virus. These grounds are summarised in the so-called "postulates of Koch," which are not, however, to be accepted as hard and fast rules. They must be construed in the light of the knowledge of disease as it exists at the time being, but modifications should be made with the greatest caution. It may be said to be certain that the number of mistakes which have been made is in direct proportion to the amount of deviation from Koch's principles.

THE EVIDENCE UPON WHICH A VIRUS MAY BE ASSOCIATED WITH THE CAUSATION OF AN INFECTIVE DISEASE.

So far as we have been able to discover, Koch did not tabulate any definite "laws" such as are often quoted at the present day.

Prof. William Bulloch has called our attention to Friedrich Loeffler's 'Vorlesungen über die geschichtliche Entwickelung der Lehre von den Bakterien.' (1) Loeffler, indeed, refers to "postulates" . . . "Constanter Nachweis, Isolirung und Prüfung der isolirten Organismen, . . . " but these are not Koch's postulates, but Henle's, formulated thus crisply by Loeffler. Henle, in his 'Pathologische Untersuchungen,' (2), says: "If one finds living motile animalcules . . . in the contagious material, it is possible . . . that they are there accidentally, if the material has been exposed for some time to the air. Even if they are found constantly in the contagious material and inside the body, it is possible . . . that they are merely parasitic elements, which develop in the fluid and may be of diagnostic importance, without being the active principle, as some still say of the spermatozoa in the semen. The only way to decide whether one is the

active principle . . . is to isolate the spermatozoa, seminal fluid, contagious organism and contagious fluid and test the power of each . . ."

This is an early and interesting deduction from purely theoretical considerations, and, indeed, contains the nucleus upon which Koch worked.

In 1878 Koch (3) stated in italics that the parasitic nature of a disease can only be certain when "the parasitic micro-organisms are to be found in all cases of the disease in question, when, further, they are demonstrable in such numbers and distribution that all symptoms of the disease are thus explicable by them, and, finally, when a morphologically well-characterised micro-organism is established as a parasite in every individual traumatic infective disease."

In 1884, having in the meantime discovered the method for the pure cultivation of bacteria, he is more explicit (4). To establish that a bacterium is the cause of tuberculosis, it is necessary, "first, to determine whether formed elements, which could neither belong to the constituents of the body nor be derived from them, are present in the lesions. . . . Whether they show any sign of possessing independent life . . . It must be shown that the parasites directly produce the disease. To obtain this proof, it is necessary to isolate the parasites . . . then to produce the disease anew with all its characteristic features by introducing the parasites alone into a healthy organism."

These are the clear principles upon which Koch worked and which are now referred to as his "postulates."

Thus to be established as the cause of an infective disease, a virus should fulfil all of three main conditions:

(1) *The virus should be capable of being recognised in a large proportion of the cases of the disease, preferably in relation to the chief lesions.*

In practice it is obviously idle to demand that the virus shall be found in every instance of a disease. The varying skill of the investigator, serious technical difficulties or, again, lack of time or facilities may make it impossible to demonstrate a special character, such as the presence of a virus, in all of a large series of cases.

Further, when the relation of a virus to a disease is under consideration, it must, of course, be understood that a "disease" in common parlance is a clinical syndrome more or less accurately recognised, and that such a syndrome is not necessarily due to a single cause. It may be recalled that the term "general paralysis" is still applied to conditions which are not syphilitic, and therefore it cannot be expected that the syphilitic virus will be found in all cases of "general paralysis" diagnosed by persons who are not familiar with the modern ætiological classification of diseases. It is only with increasing knowledge due to the close study of cases of proved ætiological similarity that those signs or symptoms are at length recognised, which indicate clinically the type of disease in which the specific virus should be demonstrable.

It is more convincing evidence of cause and effect if a virus is found alone in intimate relation to a chief or characteristic lesion of a disease; thus the meningococcus in the cerebrospinal fluid is of more obvious ætiological importance than even the pneumococcus in the pneumonic lung, the latter being often associated with large numbers of other bacteria. Koch held that bacteria should be present "in such numbers and distribution that all the symptoms of the disease may be thus explained," but it should be understood that "such numbers" does not necessarily imply large numbers, nor "distribution" a concentration in the lesions. The chief characteristic lesion of a disease may be due to toxins emanating from a small quantity of a virus situated at a distance, viz. the nervous lesions of diphtheria or tetanus.

The demonstration of the virus may be (*a*) by reproduction of the disease in other animals, or (*b*) by visible demonstration or cultivation.

(*a*) This method of demonstration is particularly applicable to viruses which are "invisible" or have not been seen. Thus the syphilis virus, before the discovery of *S. pallida*, was shown to be present in chancres by the reproduction of these lesions in animals with material taken from chancres, or the virus of typhus is known to be present in the blood of patients owing to the infective action of this blood upon guinea-pigs.

(*b*) By staining or by other methods the virus should be demonstrated as an abnormal structure of characteristic appearance in the lesions of the disease.

The appearance of such a structure may be so abnormal and may bear such a relation to certain features of a disease that its mere demonstration has been accepted as indicating the cause of a disease. Thus *B. lepræ* has not been certainly cultivated nor has it been conclusively proved capable of transmitting leprosy, but it has nevertheless been accepted as the cause of this disease.

Some viruses are present in lesions in such small quantities that a visible demonstration is impracticable; in such cases—for instance, in sporothricosis —an abnormal structure may be grown and the disease ascribed to it with some degree of probability.

If a virus can be demonstrated to be present in the lesions of a disease with relative frequency, its ætiological importance may be accepted provisionally in spite of the fact that it may also be present in other parts of the body where no obvious lesion exists, or indeed, in spite of the fact that it may often be found in association with other diseases or in healthy persons. The idea that a virus occasions disease whenever introduced into the body is no longer tenable. The development of a disease depends upon two factors, namely, the introduction of the virus in a suitable form or under suitable conditions, and the susceptibility of the individual; thus pneumococci, streptococci, meningococci and other bacteria may be present in many persons who are healthy, and yet this fact does not detract from the ætiological importance of these bacteria in the diseases ascribed to them. We may further instance the fact that

Amœba histolytica is accepted as the cause of amœbic dysentery although they are commonly found in the stools of healthy persons who have never had dysentery.

On the other hand, a pathogenic virus may be found in diseases other than that with which it is chiefly associated. For instance, pneumococci are frequently present in the lungs in phthisis although they are not the cause of the disease. They are "secondary infectors," but this does not weaken their claim to be the prime cause of lobar pneumonia.

The ascribing of a disease to a virus merely upon the optical or cultural demonstration of its presence in the lesions is, of course, always dangerous for the reason, already noted, that many viruses are commonly present in the healthy body as "saprophytes," and are thus available to take part acutely or mildly in a secondary infection. No doubt in some cases the deduction has been correct, but in others very questionable—for instance, the relation of streptococci to rheumatic fever. The history of the discovery of the causation of disease is strewn with errors due to these hasty conclusions, a recent example being the description of *B. suipestifer* as the cause of hog-cholera—a condition now known to be due to an invisible virus.

(2) *The virus should be shown to be living.*

The causative virus of an infective disease must be proved to be living and not merely a poisonous or irritant substance incapable of propagation in series. Thus, while various pollens may be accepted as the virus of hay-fever, this disease is not infective and the virus has no power of direct spread to a healthy individual. It is no more an infective agent than egg-white is infective when it happens to be swallowed by a susceptible person.

The characteristic of life, meaning the power of indefinite propagation under suitable conditions, may be demonstrated in two ways:

(1) By passage of the virus through a series of animals.

(2) By a similar passage through a series of generations in artificial culture.

The success of these methods of demonstration depends, of course, upon the discovery of a suitable animal or a suitable medium in which the virus will grow. We have already referred to the fact that the virus of typhus will propagate itself through a series of guinea-pigs, while rabies has been carried for years through innumerable rabbits in this manner. On the other hand, actinomycosis has not yet been proved to be a living virus by passage through animals because, although it is known to be pathogenic to bovines, the precise method for successful transmission has not yet been discovered.

Similarly, with artificial cultures many viruses may be propagated *ad infinitum*, but in other cases the essential medium or other condition for success is not yet available.

The determination of the fact of culture in series is not always easy. Twort (5), for instance, described a property which he had found in vaccine

lymph which had the effect of killing and dissolving certain cocci which he had isolated from the lymph. This property was capable of being carried indefinitely in series through suspensions of the cocci and thus gave the impression of being a living virus capable of growth. There is, however, more reason for supposing that the property is an autolytic ferment derived from the cocci themselves. When minute quantities are inoculated into a suspension of the cocci, these are dissolved with the liberation of more ferment; this "culture," upon being transferred, repeats the process, thus simulating growth and life. Twort's phenomenon, upon being rediscovered by d'Herrelle (6), was ascribed to a filter-passing organism—"microbe bactériophage."

(3) *The virus should be capable of reproducing the disease in other animals.*

The final criterion by which a virus may be adjudged to be the cause of a disease is its power of reproducing this disease when introduced intentionally into a healthy animal.

The inoculation should, of course, be carried out with a *pure* culture of the virus, but if it is not capable of being cultivated other methods must be found to ensure that the virus inoculated is a *pure* virus and not contaminated with some other which has been overlooked. Thus the association of malaria parasites with malaria was proved by the purification of the virus by passage through the mosquito before it was inoculated into man.

Further, it is not enough to show by inoculation that a pure virus is capable of producing a disease; it must be shown by passage that the disease produced is itself infective and due to a living virus and not to the irritant action of its constituents. It has, for instance, been claimed that a gonorrhœal infection can be produced by inoculating gonococci into the anterior chamber of the eye but, as Mezincescu and Holban (7) pointed out, this is not an infection, because the condition cannot be reproduced by transferring the pus into the eye of another rabbit. No gonococci are demonstrable in the discharge and, further, a similar lesion can be produced with dead gonococci. Thus the ophthalmitis is not a gonococcal infection in the rabbit but a non-infective lesion due to an inanimate poison.

Great difficulties sometimes arise in establishing the fact of a successful transmission of a disease. Thus, it is not to be expected that all diseases in man will be capable of being typically reproduced in animals. Obviously the outstanding symptoms of a disease in man may be due to anatomical features which animals do not possess, or may be due to methods or routes of infection to which animals are not susceptible or are not exposed. A virus supposed to be the cause of a particular illness in man may be introduced into an animal and may cause disease or death, but the essential matter to determine is whether this disease is the same disease as that which it is supposed to have caused in man.

This is a question which can seldom be settled by noting the clinical symptoms, because animals are little adapted to this method of examination. It must be settled by pathological methods.

Now, the gross pathological features of a disease are very largely governed by the method of infection and the susceptibility of the individual. Bubonic plague following infection through the skin of the leg has little in common with pneumonic plague derived from the air-passages. Similarly typhoid in the rabbit is quite different according as the virus is introduced into the peritoneal cavity or, suitably, into the stomach. The latter route reproduces the features of typhoid in man (Besredka (8)), the former does not—a different syndrome is produced.

The virus of syphilis in man produces a generalised disease, but in the rabbit the lesion is practically confined to the point of inoculation owing to the different susceptibility of this animal.

In general it may be supposed that the virus from every human infective disease *could* reproduce a closely similar disease in animals if it were always known *how* to introduce it or into what *species* of animal to introduce it. If the conditions were known under which the meningococcus, for instance, established an infection in man, it might be possible to produce meningitis in animals. Since this is not yet known meningitis cannot be produced, although peritonitis can be demonstrated in young guinea-pigs.

Thus in many cases the experimenter must perforce be content to produce in animals, not the characteristic disease of man, but another characteristic disease of animals, and he has to determine by microscopic methods or by analogy or by other methods whether this disease which he produces is comparable to the natural disease in man—in other words, whether the virus, supposed to be the cause of a disease in man, has in fact reproduced it in animals.

The method of demonstrating the existence of immune reactions in infected animals which are similar to immune reactions found in cases of human disease is often used to suggest the presence in both cases of the same virus. This is quite legitimate, but it is certainly not legitimate to assume that the virus which is present in both is the cause of the same fundamental disease in both. For instance, a *proteus* bacillus may be isolated from cases of typhus, and upon injection into an animal may produce agglutinins which are similar to agglutinins detectable in the blood of a typhus patient. From this it may be deduced that the case of typhus is infected by the *proteus* bacillus, but not that this bacillus is the cause of typhus. This disease is known to be due to another virus. The discovery of these immune reactions merely suggests an infection, which may be secondary, and cannot be accepted as proof of a successful transmission of a disease.

Having thus considered the modern application of Koch's criteria, their relation to research upon the ætiology of influenza will now be reviewed.

(*To be concluded.*)

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FIG. 1.—*Streptococcus mucosus.*

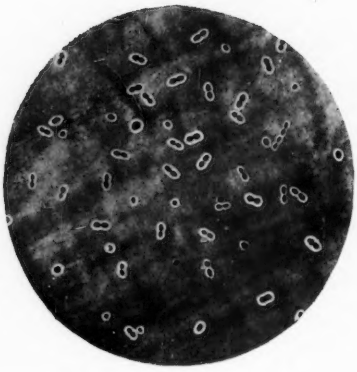

FIG. 2.—Pneumococcus.

FIG. 3.—Meningococcus.

FIG. 4.—*Staphylococcus aureus.*

TECHNIQUE.

TECHNIQUE FOR THE DEMONSTRATION OF THE CAPSULES OF BACTERIA.

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THE following is a simple and rapid method for examining an organism for the presence of capsules.

It is applicable to organisms in culture or in cerebro-spinal fluid or sometimes in thin pleural exudates, but cannot be used satisfactorily when much pus or blood is present.

METHOD:

Make a film of the organism as for ordinary staining.
Dry by moderate heat.
Spread a film of Indian ink upon the dried film. Higgins' waterproof Indian ink is recommended.
Dry the ink in the hot air over a bunsen burner.
Stain 1 to 2 minutes in hot gentian violet or carbol-gentian violet.
Wash off the superfluous stain under the tap and add Gram's iodine for a minute.
Blot dry.

The above procedure gives an intensely black organism in the middle of a perfectly transparent capsule and makes it possible to detect the slighter degrees of capsule development.

The following points should be noted:

(1) A good homogeneous background of ink is necessary: this is impossible in pus and is often interfered with by other fluids such as broth, so that the best results are obtained by emulsifying in water a loopful of a slope culture.

(2) Alcohol fixation of the film before spreading the ink gives poor results as it leads to deposition of the ink on the capsules.

(3) The staining should be as intense as possible, since if the organism is not perfectly opaque to light its transparent margin may give the false impression of a capsule. This is seen in any dead and poorly staining

organisms which may be present; they appear as white spaces or more often as slightly coloured spots with white margins.

(4) On keeping, the black iodine-treated specimens fade to a violet, which, however, still shows the capsule satisfactorily as a rule; if necessary they can be washed in xylol and alcohol and re-stained without damage to the ink film.

The plate shows four organisms with different degrees of capsule development.

The pneumococcus is not satisfactorily dealt with by the above technique since its capsule is relatively easily stained and therefore does not show up as a white area: in this case the specimen should be washed in alcohol after the iodine as in Gram's stain.

The staphylococcus shows no appreciable capsule by this method. Occasionally a specimen is found with a barely visible bright line at the edge, but this is on one side only and appears to be due to slight contraction or shifting of the organism.

It is generally recognised that Indian ink gives the correct outline of organisms and this applies to the capsular outline, but by the use of too thick a film the organism may be partly covered up and the capsule obscured.

Shrinkage during the staining process is inappreciable; there may be slight shrinkage or displacement of a few of the organisms but this is insufficient to give the effect of a capsule.

A NEW MEDIUM FOR THE GROWTH OF *B. INFLUENZÆ.*

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(*A report to the Medical Research Committee.*)

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WHEN Matthews introduced his trypsin-digested medium for the growth of *B. influenzæ*, I found it inconvenient to use human blood and had recourse to ox and sheep's blood. Trypsin, however, does not digest these bloods to the same extent as human; even when the digest has been maintained at 37° C. for a number of days, there is always a precipitate of proteins when it is mixed with agar.

The effect of peptic digestion was then tried, and it was found that such a digest even after only 30 minutes at 55° C. could be rendered faintly alkaline and mixed with agar without any precipitate occurring.

This peptic digest grows *B. influenzæ* in the same copious manner as does Matthews' medium, and it has the advantage of being much more rapidly prepared and more constant in action. Sterility of the initial reagents is of no consequence. Samples of the digest have been kept at room temperature for over twelve months without undergoing deterioration.

The following is the manner of preparation:

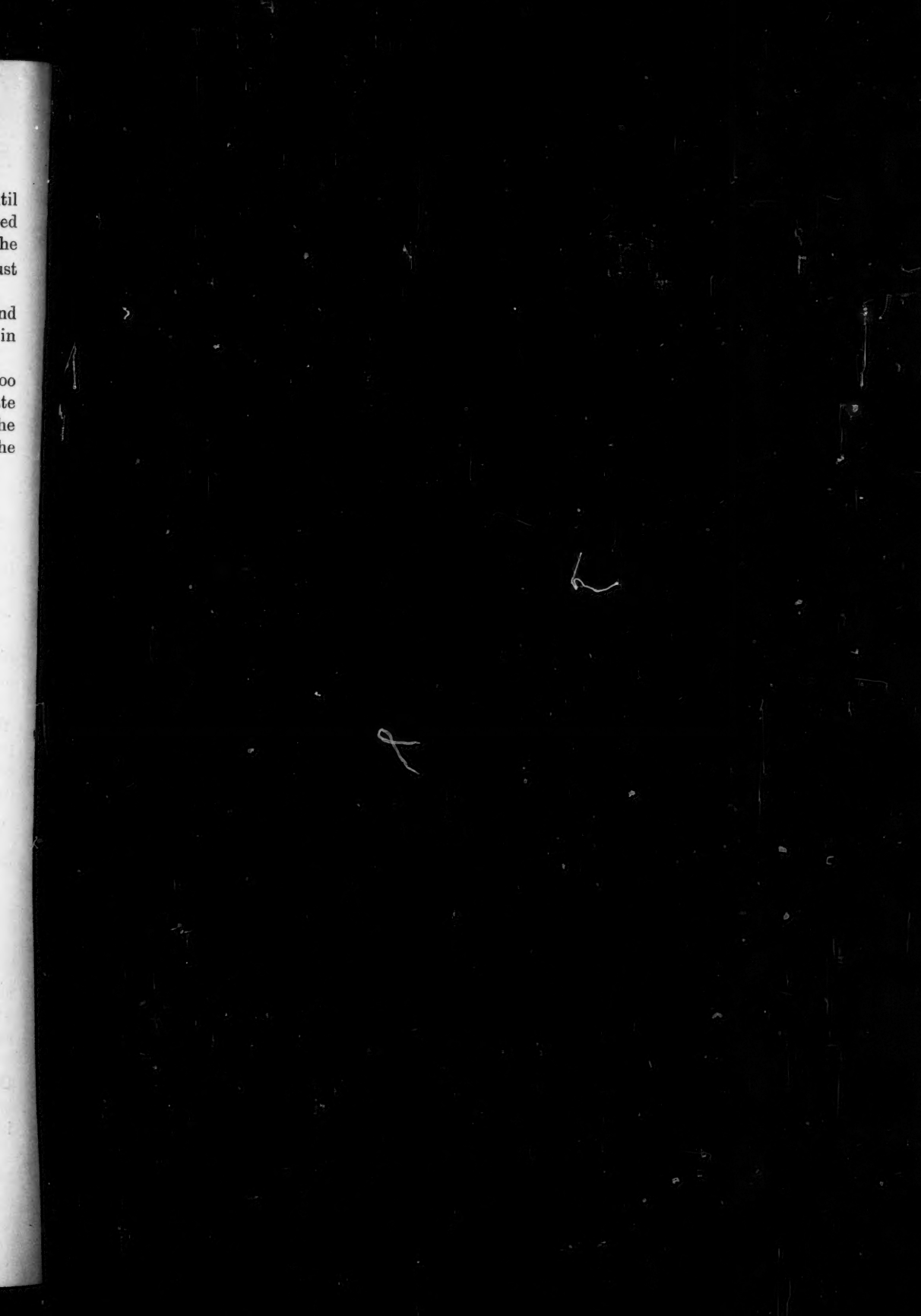
Into a 250 c.c. bottle, fitted with a well-ground glass stopper, place in the indicated order—

(1) 150 c.c. saline solution, not distilled water.
(2) 6 c.c. pure HCl.
(3) 50 c.c. defibrinated sheep's blood.
(4) 1 grm. pepsin, B.P., granulated.

Shake to dissolve. Place in the water bath at 55° C. for 2 to 24 hours, shaking occasionally at first. The exact time is unimportant. Then add 12 c.c. of 20 per cent. NaOH, accurately prepared, and test the reaction with cresol red (·02 per cent. solution). If this dye does not give a colour of permanganate, continue adding 20 per cent. NaOH until this point is reached (P_H 7·6). For the purpose of testing, the samples are removed from the bottle with a sterile pipette and diluted with distilled water until the colour is light enough to show the change on the addition of a few drops of cresol

red. When the correct point is reached add pure HCl drop by drop until cresol red gives practically no change of colour, but phenol red gives red (P_H 7·0 to 7·2). Finally add 0·25 per cent. of chloroform, slightly grease the stopper and shake to dissolve the chloroform. Protect the bottle from dust with a paper cap.

For use, shake the bottle, remove the material with a sterile pipette and add it directly to melted agar in a strength of 2 to 5 per cent. or to broth in the same proportions. It is not necessary to remove the chloroform.

The correct adjustment of the reaction is essential; if the bottle is left too alkaline the contents will rapidly deteriorate. On standing a slight precipitate of hæmatin takes place and the bottle should be shaken before use, but if the reaction is too acid the precipitate will be excessive and the quality of the medium will suffer accordingly.

Nutrient agar base